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Additions to the taxonomy of plant pathogenic/foliicolous *Mycosphaerellaceae* from the Brazilian Atlantic Rainforest

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Magister Scientiae

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Dissertation submitted to the Plant Pathology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Olinto Liparini Pereira

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Assent:

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To my parents.

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ABSTRACT

NOGUEIRA, Pedro Thiago Santos, M.Sc., Universidade Federal de Viçosa, February, 2025. **Additions to the taxonomy of plant pathogenic/foliicolous *Mycosphaerellaceae* from the Brazilian Atlantic Rainforest**. Adviser: Olinto Liparini Pereira.

The Atlantic Forest, a biodiversity hotspot in Brazil, is widely recognized for its remarkable biological richness. However, fungal diversity, particularly plant pathogens, remains unclear. Among these fungi, the family *Mycosphaerellaceae* is highly diverse, comprising numerous genera and species that cause diseases in native plants and in cultivated plants. Recent studies have demonstrated that morphology alone is insufficient to differentiate between certain genera within this family, revealing the existence of cryptic taxa. Despite previous studies in Brazil, there is a significant gap in research that specifically focuses on the Atlantic Forest. This study aimed to describe fungi of the *Mycosphaerellaceae* family associated with plants in the Atlantic Forest using a polyphasic approach. Plant samples with leaf spot symptoms were collected from forest fragments at the Federal University of Viçosa, cercosporoid fungi were isolated and cultured in the laboratory. Diagnostic characters include the production of one sporodochium per lesion in *Mycosphaerellaceae* gen. *et*. sp. 1, the production of long catelunnate spores in *Mycosphaerellaceae* gen. *et*. sp. 2, and the production of spores by thallic conidiogenesis in *Mycosphaerellaceae* gen. *et*. sp. 3. Using a polyphasic approach, three new fungal genera and three new fungal species were identified and described, supported by molecular, morphological, and ecological data. This study significantly advances our understanding of fungal diversity in the Atlantic Forest.

Keywords: *Cercospora;* ; Cercosporoid; Multi-gene phylogeny; New taxa; Biodiversity

RESUMO

NOGUEIRA, Pedro Thiago Santos, M.Sc., Universidade Federal de Viçosa, fevereiro de 2025. **Adições à taxonomia de *Mycosphaerellaceae* fitopatogênicos/folícolas da Mata Atlântica Brasileira**. Orientador: Olinto Liparini Pereira.

A Mata Atlântica, um hotspot de biodiversidade no Brasil, é amplamente reconhecida por sua notável riqueza biológica. No entanto, a diversidade de fungos, particularmente patógenos de plantas, permanece obscura. Entre esses fungos, a família *Mycosphaerellaceae* é altamente diversa e compreende vários gêneros e espécies que causam doenças em plantas nativas e também em plantas cultivadas. Estudos recentes demonstraram que a morfologia por si só é insuficiente para diferenciar certos gêneros dentro desta família, revelando a existência de táxons crípticos. Apesar de estudos anteriores no Brasil, há uma lacuna significativa na pesquisa que se concentra especificamente na Mata Atlântica. Este estudo teve como objetivo descrever fungos da família *Mycosphaerellaceae* associados a plantas na Mata Atlântica usando uma abordagem polifásica. Amostras de plantas com sintomas de manchas foliares foram coletadas de fragmentos florestais na Universidade Federal de Viçosa, fungos cercosporoides foram isolados e cultivados em laboratório. Utilizando uma abordagem polifásica, três novos gêneros e três novas espécies fúngicas foram identificadas e descritas, apoiadas por dados moleculares, morfológicos e ecológicos. Como caracter diagnóstico podemos citar a produção de um esporodóquio por lesão no *Mycosphaerellaceae* gen. *et*. sp. 1, produção de longos esporos catelunados em *Mycosphaerellaceae* gen. *et*. sp. 2, e produção de esporos por conidiogênese tálica no *Mycosphaerellaceae* gen. *et*. sp. 3. Este estudo aumenta significativamente nossa compreensão da diversidade fúngica na Mata Atlântica.

Palavras-chave: *Cercospora*; Cercosporoide; Filogenia multigênica; Novos taxa; Biodiversidade

SUMMARY

INTRODUÇÃO GERAL

O Brasil é um país com proporções continentais e mega-diverso, com 10-17,6% de toda a diversidade do planeta (Lewinsohn; Prado, 2005). No país há seis biomas, destes dois são considerados hotspot, o Cerrado e a Mata Atlântica, sendo o último o mais devastado com cerca de 11,4-16% de sua cobertura original, além de ter diversas espécies ameaçadas de extinção, o que eleva a necessidade de estudos sobre esse bioma (Ribeiro *et al.*, 2009).

Um desafio mundial, e mais acentuado em países em desenvolvimento, é conhecer a diversidade de microrganismos (Pylro *et al.*, 2014). Especificamente para os fungos, estima-se que haja 2,5 milhões de espécies, contudo após mais de 250 anos de estudos conhecemos cerca de 150.000 espécies, e apesar do crescimento da micologia e aumento dos estudos, ainda estamos em uma média 2.000 espécies descritas anualmente (Niskanen *et al.*, 2023). Levando em consideração esta média, para que pudéssemos descrever toda a diversidade fúngica seria necessário cerca de um milênio, o que se torna preocupante já que os ambientes naturais estão à mercê da ação antrópica.

Os fungos estão comumente associados às plantas, seja como endófitos, micorrizas ou fitopatógenos. A Mata Atlântica abriga por volta de 3,4 mil espécies de fungos (Flora e Funga do Brasil - Fungos, 2025), e cerca de 20 mil espécies de plantas, porém a maioria dessas espécies de plantas ainda carece de algum estudo sobre sua micobioma (Ruschel; Nodari; Moerschbacher, 2007). Os fungos fitopatogênicos compreendem uma parte significativa deste micobioma. Entre os grupos mais diversos de fungos fitopatogênicos estão os da família *Mycosphaerellaceae*.

Os fungos da família *Mycosphaerellaceae* derivam seu nome do gênero *Mycosphaerella*. O nome *Mycosphaerella* Johanson tem origem em *Sphaerella*, um gênero descrito por Saccardo em 1882 para acomodar fungos no gênero *Sphaeria* (fungos com peritécios esféricos) que apresentavam hialodidimósporos. No entanto, devido à existência de um gênero de algas e um gênero de plantas com o mesmo nome e em conformidade com o código de nomenclatura da época, o nome *Sphaerella* foi substituído por *Mycosphaerella* (Aptroot, 2006). Outras características distintivas de *Mycosphaerella* incluem a presença de pseudotécios com perífises ostiolares e tecido interascal na maturidade. Atualmente, devido a mudanças nas

regras de nomenclatura fúngica, o nome de sua morfologia assexuada, *Ramularia*, foi escolhido como o nome atual do gênero (Wingfield *et al.*, 2012). Apesar disso, *Mycosphaerella* sensu lato está associado a outros gêneros assexuados, demonstrando que suas características definidoras não são exclusivas de *Ramularia* (Aptroot, 2006; Crous, 2009; Videira *et al.*, 2017).

Apesar das diferenças morfológicas limitadas na fase sexuada, a fase assexuada em *Mycosphaerellaceae* é altamente diversa. Durante muito tempo, o conceito morfológico de espécie baseado nessas características foi o principal critério para distinguir gêneros desta família. Entre as morfologias assexuadas, estão representados tanto os hifomicetos quanto os coelomicetos. Os gêneros foram separados principalmente com base em características como a presença ou ausência de pigmentação nas estruturas conidiogênicas, a disposição e ramificação dos conidióforos, a localização das células conidiogênicas, o tipo de proliferação, o tipo de cicatriz (lócus conidiogênico), a formação, forma e septação dos conídios (Braun, 1995; Chupp, 1953; Crous *et al.*, 2000, 2009c; Deighton, 1967, 1973, 1976, 1979; Ellis, 1971). No entanto, espécies com morfologias intermediárias ou que apresentavam mais de um tipo de conidiogênese sempre representaram um desafio para a taxonomia desse grupo (Crous; Braun, 2003).

Outro problema significativo na taxonomia do grupo foi o uso do conceito ecológico de espécie, no qual se acreditava que cada hospedeiro possuía um patógeno fúngico único. Essa abordagem contribuiu para o grande número de espécies descritas (Aptroot, 2006; Chupp, 1953; Corlett, 1991, 1995; Crous *et al.*, 2000). A aplicação deste conceito revelou-se variável: em alguns casos, a especificidade do hospedeiro é um fator decisivo para a delimitação de espécies, enquanto em outros não (Groenewald *et al.*, 2013; Videira *et al.*, 2015).

Com o avanço e a redução de custos das tecnologias de sequenciamento, a taxonomia dos fungos também evoluiu, o conceito de espécie filogenética ofuscou progressivamente os conceitos morfológicos e ecológicos. As primeiras tentativas foram realizadas utilizando sequências de ITS de um pequeno número de espécimes, demonstrando que *Mycosphaerella* e seus anamorfos formavam um grupo monofilético (Crous *et al.*, 2000). No entanto, à medida que o tamanho das bases de dados aumentou e filogenias multigênicas foram construídas, ficou evidente que *Mycosphaerellaceae* não era um grupo monofilético, e tanto as fases sexuadas quanto as assexuadas precisavam de revisão (Bensch *et al.*, 2012; Crous

et al., 2009a, 2009b, 2009c). O primeiro passo crítico foi a epitipificação da espécie-tipo da família para definir seus limites. Foi demonstrado que *Mycosphaerella punctiformis* tinha *Ramularia endophylla* como sua fase assexuada, delimitando assim *Mycosphaerella* sensu stricto. As demais espécies semelhantes a *Mycosphaerella* foram posteriormente realocadas para outros gêneros em estudos subsequentes (Verkley *et al.*, 2004). O segundo passo essencial na taxonomia do grupo foi a reorganização interna dos gêneros e espécies da família, que também se mostraram não monofiléticos. Além disso, muitas das características morfológicas anteriormente utilizadas para classificação mostraram-se incongruentes com as análises filogenéticas (Júnior *et al.*, 2014; Videira *et al.*, 2015, 2016).

No Brasil esses fungos foram estudados por diversos pesquisadores em diferentes períodos, e em diferentes biomas (Batista; Silva, 1953; Braun; David; Freire, 1999; Hernández-Gutiérrez; Dianese, 2009; Muller; Chupp, 1934; Viégas, 1941). Contudo ainda após a era da biologia molecular poucos trabalhos foram feitos para estabelecer as relações filogenéticas entre as espécies (Guatimosim, 2016; Silva *et al.*, 2016).

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RESEARCH ARTICLE

According to the guidelines of Fungal Systematics and Evolution

Additions to the taxonomy of plant pathogenic/foliicolous Mycosphaerellaceae from the Brazilian Atlantic Rainforest

Additions to the taxonomy of plant pathogenic/foliicolous *Mycosphaerellaceae* from the Brazilian Atlantic Rainforest

ABSTRACT

The Atlantic Forest, a biodiversity hotspot in Brazil, is widely recognized for its remarkable biological richness. However, the diversity of fungi, particularly plant pathogens, remains unclear. Among these fungi, the family *Mycosphaerellaceae* is highly diverse and comprises numerous genera and species that cause diseases in in native and in cultivated plants. Despite previous studies in Brazil, there is a significant gap in research that specifically focuses on the Atlantic Forest. Recent studies have demonstrated that morphology alone is insufficient to differentiate between certain genera within this family, revealing the existence of cryptic taxa. This study aimed to describe fungi of the family *Mycosphaerellaceae* associated with plants in the Atlantic Forest using a polyphasic approach. Leaves with leaf spot symptoms were collected from forest fragments at the Universidade Federal de Viçosa, cercosporoid fungi were isolated and cultured in the laboratory, and DNA was extracted and subjected to PCR amplification of Internal transcribed spacer (ITS), large subunit ribosomal (LSU), and RNA polymerase second largest subunit gene (*RPB2*) markers. As a result, three new genera (*Mycosphaerellaceae* gen*. 1*, *Mycosphaerellaceae* gen*. 2*, and *Mycosphaerellaceae* gen*. 3*) and three new species (*Mycosphaerellaceae* sp*. 1*, *Mycosphaerellaceae* sp*. 2*, and *Mycosphaerellaceae* sp*. 3*) were identified, supported by phylogenetic data, morphological characteristics, and ecological information from their hosts. Diagnostic characters include the production of one sporodochium per lesion in *Mycosphaerellaceae* gen. *et*. sp. 1, the production of long catelunnate spores in *Mycosphaerellaceae* gen. *et*. sp. 2, and the production of spores by thallic conidiogenesis in *Mycosphaerellaceae* gen. *et*. sp. 3. This study significantly enhances our understanding of fungal diversity in the Atlantic Forest.

Keywords: *Cercospora*, Cercosporoid; Multi-gene phylogeny; New taxa; Biodiversity

INTRODUCTION

The Atlantic Forest is a biome in South America, with 92% of its area located in Brazil (de Lima *et al.*, 2020). This biome is considered a biodiversity hotspot, being one of the most diverse and most degraded biomes in the world (Myers *et al.*, 2000). Approximately 20 000 plant species are found in the Atlantic Forest, with around 8 000 of these being endemic (Ruschel; Nodari; Moerschbacher, 2007). Associated with these plants is a great diversity of fungal species, including mycorrhizae, endophytes, and plant pathogens (Pinho *et al.* 2013, Freitas *et al.* 2020, Condé *et al.* 2025). Plant pathogenic fungi can be host-specific and comprise a vast diversity that remains to be explored, particularly in a biome like the Atlantic Forest, which has a high diversity of endangered plants (Crous 2009, Vialle *et al*. 2013).

Mycosphaerellaceae fungi constitute a mega-diverse family with 153 accepted genera (MycoBank, accessed 18 Nov. 2024) and in 'The 2024 outline of fungi and fungus-like taxa' there were 135 accepted genera (Hyde *et al.* 2024). Some of these genera are highly diverse, such as *Cercospora*, *Passalora*, *Pseudocercospora*, *Ramularia*, and *Septoria*, with 3 629, 784, 1 902, 1 259, and 3 333 names, respectively, recorded in the MycoBank (accessed 18 Nov. 2024). Although primarily recognized as plant pathogens, this family also includes epiphytic, saprophytic, endophytic, fungicolous, and lichenicolous species (Braun *et al*. 2013, Videira *et al*. 2017, Navarro-Rosinés & Roux 2017). Additionally, *Mycosphaerellaceae* is cosmopolitan, occurring across all continents (Crous & Braun 2003, Navarro-Rosinés & Roux 2017).

Some genera within the *Mycosphaerellaceae* family rank among the most significant plant pathogens globally primarily because of their asexual forms. *Cercospora*, for instance, has been recognized as one of the 100 most frequently cited fungi worldwide (Bhunjun *et al.* 2024). This prominence is due to various factors, one of which is the broad range of plants these fungi affect, including hosts in the *Pteridophyta*, *Gymnospermae*, *Poaceae*, *Fabaceae*, and *Asteraceae*, among dozens of others (Crous & Braun 2003, Braun *et al.* 2013, Braun *et al.* 2015). Additionally, several species cause significant crop losses, such as *Cercospora beticola*, which causes Cercospora Leaf Spot of Sugar Beet; *Pseudocercospora griseola*, which causes Common Bean Angular Leaf Spot; *Fulvia fulva*, which causes Leaf Mold of Tomato; and *Nothopassalora personata*, which causes Peanut Late Leaf Spot (Oliveira *et al.* 2004, Thomma *et al.* 2005, Secor *et al.* 2010, Júnior *et al.* 2014, Giordano *et al.* 2021). Finally, another critical factor is quarantine regulations, as some of these species are not cosmopolitan and can cause highly destructive diseases, such as *Pseudocercospora ulei*, the causal agent of Leaf Blight of Rubber, which is absent in major rubber-producing countries (Júnior *et al.*, 2014).

In Brazil, the situation is similar, many species are economically important (e.g., *Pseudocercospora griseola*, *Cercospora coffeicola*, *Septoria glycines*), including some that are subject to quarantine regulations (e.g., *Pseudocercospora fijiensis*) (Kimati *et al.*, 1997). Additionally, the diversity of these species has been studied by numerous authors (Viégas 1941, Batista & Silva 1953, Crous *et al.* 1997, Crous & Câmara 1998, Braun *et al.* 1999, Crous *et al.* 1999a, Braun & Freire 2002, Braun & Freire 2004, Pereira & Barreto 2005, Braun & Freire 2006, Parreira *et al.* 2014; Silva *et al.* 2016). The first major studies on the family were focused primarily on the Cerrado biome (Inacio & Dianese 1999; Furlanetto & Dianese 1999; Pereira *et al.* 2006; Dornelo-Silva *et al.* 2007; Dianese *et al.* 2008; Hernández-Gutiérrez &

Dianese 2008, Hernández-Gutiérrez & Dianese 2009, Hernández-Gutiérrez *et al.* 2014, Hernández-Gutiérrez *et al.* 2015). Subsequently, this diversity was also explored in the Atlantic Forest (Soares & Barreto 2005, Soares *et al.* 2006, Pereira & Barreto 2006, Pereira *et al.* 2007, Rocha *et al.* 2008, Firmino *et al.* 2013, Parreira *et al.* 2014).

In general, asexual morphs are more informative than sexual morphs, because *Mycosphaerella*-like morphology in the broad sense is present in various hyphomycete genera within the family (Aptroot 2006, Crous 2009, Videira *et al.* 2017). Currently, these asexual morphs can be primarily distinguished as *Cercospora*-, *Passalora*-, *Pseudocercospora*-, *Pseudocercosporella*-, *Ramularia*-, *Septoria*-, and *Zasmidium*-like, and the hyphomycete morphs being known as cercosporoids (Videira *et al.*, 2017). Although morphology can provide clues for species identification, it alone is insufficient since these forms are not always monophyletic (Videira *et al.* 2016, 2017, Yadav *et al.* 2023). Therefore, this study aimed to describe new *Mycosphaerellaceae* taxa associated with plants in the Atlantic Forest based on phylogeny, morphology and ecological features.

MATERIAL AND METHODS

Collection, isolation, and storage

Plants presenting leaf spots were collected in forest fragments and fields at the Universidade Federal de Viçosa (Viçosa, MG, Brazil). Leaf samples were taken to the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas, where they were examined under a stereomicroscope to observe fungal structures. Those with cercosporoid leaf spot symptoms were dried in a plant press for herborization. Cercosporoid fungi observed in the samples were isolated directly from single conidia on potato dextrose agar (PDA) media. An adaptation of the method described by Cordeiro *et al.* (2011) was used to increase the speed of coverage of the culture medium surface and induce sporulation. A mycelium disc was macerated in 1ul of autoclaved distilled water and spread over the surface of the V8-juice agar (V8). Cultures on V8 were stored in duplicates in 2 mL microtubes containing water, 2 mL microtubes containing 10% glycerol solution at -20°C, and anhydrous silica gel at 5°C (Castellani 1939, Dhingra & Sinclair 2017). A replicate of the tubes containing glycerol solution and silica gel were deposited in the culture collection Oswaldo Almeida Drummond Collection (COAD), housed at the Universidade Federal de Viçosa. Representative specimens were deposited at the Fungarium of the Universidade Federal de Viçosa (VIC). Nomenclatural novelties and descriptions were deposited in MycoBank (www.mycobank.org).

DNA extraction, PCR amplification and sequencing

Fungal isolates were cultivated on PDA plates for 7 d at 25 °C in the dark, and total genomic DNA was extracted using the Wizard® Genomic Purification Kit (Promega) according to the protocol described by Pinho *et al.* (2013). The internal transcribed spacer 1 and 2 including the 5.8S rDNA region (ITS) and 28S Large Subunit rDNA (LSU) regions were amplified using the primers ITS5 and LR6 (Vilgalys & Hester 1990, White *et al.* 1990). The RNA polymerase second largest subunit gene (*RPB2*) region was amplified using the primers RPB2-5F2 (Sung *et al.* 2007) and fRPB2-7cR (Liu *et al.* 1999). The PCR conditions were set for an initial denaturation step at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C (ITS) for 90s, and 55 °C (*RPB2*) for 45 s, elongation at 72 °C for

30–60 s, and a final elongation step at 72 °C for 5 min. Purification and sequencing of the DNA amplicons were carried out by Macrogen, Inc. (South Korea). The sequencing data were visualized and trimmed using FinchTV software (Geospiza Inc.).

Phylogenetic analyses

A database of ITS, LSU, and *RPB2* regions for the family *Mycosphaerellaceae* was compiled using the available sequences from the GenBank database for type or reference specimens (Table 1). Alignments were independently generated for each gene using the online MAFFT V7 platform, applying the L-INS-i parameter for all genes (Katoh & Standley 2013). Manual adjustments were performed using MEGA v.11 (Tamura *et al.*, 2021). Sequence Matrix software was used to concatenate the alignments (Vaidya *et al.*, 2011).

Individual and concatenated Maximum Likelihood (ML) analyses were conducted using IQ-Tree 2.2.2.7 software (Nguyen *et al.* 2015). Ultrafast bootstrap initialization was used (Hoang *et al.* 2018) and 5 000 bootstrap replicates were performed. The MERGE function was applied in the concatenated analysis. The concatenated tree based on Bayesian Inference (BI) was constructed using MrBayes 3.2.7 on XSEDE via the CIPRES portal (Ronquist & Huelsenbeck, 2003, Miller *et al.* 2010). Model selection was performed with jModelTest2 2.1.10, using the Bayesian information criterion (BIC) as the selection criterion. Two independent runs of four Markov Chain Monte Carlo (MCMC) simulations were conducted simultaneously, each beginning with random trees for 10 000 000 generations, sampling every 500 generations. Intermediate results were saved at intervals of 1 000 generations, and analytical comparisons between the two runs were made every 500 generations, with 25% of the initial trees discarded at each diagnostic check. The remaining trees were then used to calculate Bayesian posterior probability (BPP) values. The resulting trees were visualized using Figtree v1.4.4 (Rambaut 2018). DNA sequences from this study were deposited in GenBank (Table 1), and the alignments were uploaded to FigShare (in processing). Phylogenetic comparisons were made using the NCBI BLASTn tool.

Morphology

Micromorphological descriptions were made from both *in vivo* and *in vitro* materials with semi-permanent slides mounted using Shears mounting (Crous, P.W. *et al.*, 2009). For the *in vitro* descriptions, the media described by Videira *et al.* (2017) were used: synthetic nutrient-poor agar (SNA), V8-juice agar (V8), malt extract agar (MEA), and oatmeal agar (OA), incubated for 2–4 weeks at 25°C under 12 hour photoperiod (Crous, P.W. *et al.*, 2009). The spore induction method proposed by Cordeiro *et al.* (2011) was used. Microphotographs were taken using an Olympus BX53 microscope equipped with an Olympus Q-Color5 digital camera and compound differential interference contrast (DIC) illumination. Morphological features were measured (n ≥ 30) using the Olympus cellSens Dimension 1.9 software. For cultural characterization, cultures were incubated on MEA at 25°C in the dark for 3 weeks, and colony colours were assessed using Rayner's colour chart (Rayner 1970).

The morphological comparisons and discussions of the new species were performed with the phylogenetically closest species and genera, as well as with species associated with the host genera.

RESULTS

Phylogenetic analyses

Seven fungal isolates were identified: two from *Samanea inopinata* (*Fabaceae*), two from *Erythrina speciosa* (*Fabaceae*), and three from *Swietenia macrophylla* (*Meliaceae*). Phylogenetic analyses revealed three lineages distinct from any previously described within *Mycosphaerellaceae* (Fig. 1.). As a result, three new genera were described: *Mycosphaerellaceae* gen. *1* gen. nov., *Mycosphaerellaceae* gen. *2* gen. nov., and *Mycosphaerellaceae* gen. *3* gen. nov., as well as three new species, *Mycosphaerellaceae* sp. *1* sp. nov., *Mycosphaerellaceae* sp. *2* sp. nov., and *Mycosphaerellaceae* sp. *3* sp. nov.

The alignment was made with 146 sequences from 128 genera, the sizes for each gene were 559 bp for ITS (of which 212 were conserved, 318 were variable, and 237 were parsimony-informative), 749 bp for LSU (of which 505 were conserved, 228 were variable, and 170 were parsimony-informative), and 929 bp for *RPB2* (of which 311 were conserved, 599 were variable, and 532 were parsimony-informative). The evolutionary model was selected for Bayesian Inference (BI) using jModelTest2 2.1.10, and the Bayesian Information Criterion (BIC) was GTR+G+I for all three alignments. For the Maximum Likelihood (ML) analysis conducted in IQTree 2.2.2.7, the models selected were TIM2+F+R5 for ITS, SYM+I+G4 for LSU, and GTR+F+I+G4 for *RPB2*.

The isolates obtained from plants of *Samanea inopinata* formed a distinct branch from other genera in both the individual and concatenated analyses. In concatenated phylogenies, this branch was sister to the genus *Asperisporium* and was well supported in both phylogenies, reaching maximum values for Bayesian Posterior Probability (BPP) and Bootstrap Support (BS) (Fig. 1.). However, the sister-genera relationship was maintained only in the individual *RPB2* phylogeny, with a BS = 100% (Supplementary Fig. S3.). In the ITS phylogeny, *Mycosphaerellaceae* gen. *1* formed a sister branch to *Sirosporium* with a BS of 100 (Supplementary Fig. S1.). In the LSU phylogeny, *Mycosphaerellaceae* gen. *1* formed a sister branch to *Sultanimyces* and *Neocercosporidium* with a BS of 100 (Supplementary Fig. S2.). Comparing *Mycosphaerellaceae* sp. *1* COAD 3861 with closely related species, ITS sequences showed 94% identity (416/441) with six gaps to *Asperisporium caricae* CBS 130298, 96% identity (420/441) with one gap to *Sirosporium celtidis* CBS 158.25, 92% identity (403/436) with one gap to *Sultanimyces vitiphyllus* CBS 206.48, 93% identity (407/437) with two gaps to *Neocercosporidium smilacis* CBS 122888, 97% identity (421/436) with one gap to *Amycosphaerella africana* CBS 680.95, and 97% identity (421/436) with one gap to *Pantospora guazumae* CBS 130299. The LSU sequence showed 98% identity (715/727) with two gaps to *Asperisporium caricae* CBS 130298, 99% identity (716/725) with no gaps to *Sirosporium celtidis* CBS 158.25, 98% identity (709/725) with no gaps to *Sultanimyces vitiphyllus* CBS 206.48, 99% identity (717/725) with no gaps to *Neocercosporidium smilacis* CBS 122888, 99% identity (717/727) with one gap to *Amycosphaerella africana* CBS 680.95, and 99% identity (719/725) with one gap to *Pantospora guazumae* CBS 130299. For *RPB2*, *Mycosphaerellaceae* sp. *1* COAD 3861 showed 94% identity (850/907) with no gaps to *Asperisporium caricae* CBS 130298, 88% identity (738/843) with no gaps to *Sirosporium celtidis* CBS 158.25, 87% identity (742/852) with no gaps to *Sultanimyces vitiphyllus* CBS 206.48, 86% identity (749/870) with no gaps to *Neocercosporidium smilacis* CBS 122888, 92% identity (794/866) with no gaps to *Amycosphaerella africana* CBS 680.95, and 92% identity (816/889) with no gaps to *Pantospora guazumae* CBS 130299.

Similar to *Mycosphaerellaceae* gen*. 1*, *Mycosphaerellaceae* gen*. 3* formed a clade distinct from other genera in both individual and concatenated analyses. In concatenated phylogenies, the clade was sister to the genus *Pantospora* and was well supported in both phylogenies, reaching the maximum values for BPP and BS (Fig. 1.). However, similar to *Mycosphaerellaceae* gen*. 1*, the sister genus relationship was maintained only in the individual *RPB2* phylogeny with a BS of 100 (Supplementary Fig. S3.). In the LSU phylogeny, *Mycosphaerellaceae* gen*. 3* formed a sister clade to *Deightonomyces*, with a BS of 100 (Supplementary Fig. S2.). In ITS phylogeny, the node giving rise to the *Mycosphaerellaceae* gen*. 3* clade formed a polytomy with several genera (Supplementary Fig. S1.). Comparing *Mycosphaerellaceae* sp. *3* COAD 3867 with closely related species, ITS sequences showed 98% identity (430/438) with one gap with *Pantospora chromolaenae* CBS 145563, 97% identity (427/438) with three gaps with *Deightonomyces daleae* CBS 113031, 95% identity (417/438) with five gaps with *Pruniphilomyces circumscissus* CBS 145985, and 98% identity (429/437) with two gaps with *Amycosphaerella africana* CBS 680.95.The LSU sequences showed 99% identity (721/727) with two gaps to *Pantospora chromolaenae* CBS 145563, 99% identity (722/727) with two gaps to *Deightonomyces daleae* CBS 113031, 99% identity (721/727) with two gaps to *Pruniphilomyces circumscissus* CBS 145985, and 99% identity (720/727) with no gaps to *Amycosphaerella africana* CBS 680.95. For *RPB2*, Mycosphaerellaceae sp. 3 COAD 3867 showed 89% identity (778/879) with no gaps to *Pantospora chromolaenae* CBS 145563, 78% identity (686/874) with no gaps to *Deightonomyces daleae* CBS 113031, 83% identity (486/589) with no gaps to *Pruniphilomyces circumscissus* CBS 145985, and 92% identity (505/589) with no gaps to *Amycosphaerella africana* CBS 680.95.

Mycosphaerellaceae gen*. 2* formed a long and well-supported branch with maximum BPP and BS values in concatenated analyses (Fig. 1.). Nevertheless, it did not form an independet lineage to the genera *Neoramulariopsis* and *Cercosporella* in the individual ITS and LSU analyses, respectively (Supplementary Fig. S1., Supplementary Fig. S2.). In the individual *RPB2* phylogeny, *Mycosphaerellaceae* gen*. 2* formed a long well-supported branch with a BS of 100 (Supplementary Fig. S3.). Comparing *Mycosphaerellaceae* sp. *2* COAD 3865 with closely related species, ITS sequences showed 93% identity (413/442) with eight gaps to *Acervuloseptoria ziziphicola* CBS 138009, 91% identity (402/441) with 10 gaps to *Neocercosporella peristrophes* AMH 9671, 97% identity (420/435) with one gap to *Cercosporella virgaureae* CPC 11460, 97% identity (423/435) with no gaps to *Neoramulariopsis catenulata* CBS 355.73, and 98% identity (430/438) with three gaps to *Ramulariopsis gossypii* CBS 141099. LSU sequences showed 99% identity (715/727) with two gaps to *Acervuloseptoria ziziphicola* CBS 138009, 99% identity (719/725) with no gaps to *Neocercosporella peristrophes* AMH 9671, 99% identity (724/727) with no gaps to *Cercosporella virgaureae* CPC 11460, 99% identity (723/727) with no gaps to *Neoramulariopsis catenulata* CBS 355.73, and 99% identity (722/727) with no gaps to *Ramulariopsis gossypii* CBS 141099. For *RPB2*, *Mycosphaerellaceae* sp. *2* COAD 3865 showed 84% identity (744/881) with no gaps with *Acervuloseptoria ziziphicola* CBS 138009, 85% identity (749/883) with no gaps with *Neocercosporella peristrophes* AMH 9671, 85% identity (570/672) with no gaps with *Cercosporella virgaureae* CPC 11460, 82% identity (653/795) with no gaps with *Neoramulariopsis catenulata* CBS 355.73, and 83% identity (550/666) with no gaps with *Ramulariopsis gossypii* CBS 141099.

The phylogenetic analyses revealed that the three newly identified *Mycosphaerellaceae* lineages formed distinct and well-supported clades, justifying their recognition as separate genera. *Mycosphaerellaceae* gen. 1 and gen. 3 were each consistently resolved as sister groups to established genera (*Asperisporium* and *Pantospora*, respectively) in concatenated and individual phylogenies. *Mycosphaerellaceae* gen. 2, while forming a long and well-supported clade in the concatenated and *RPB2* analyses, showed phylogenetic proximity to *Neoramulariopsis* and *Cercosporella* in the ITS and LSU trees, respectively, without forming an independent lineage in those markers. Nevertheless, sequence comparisons further supported the genetic distinctiveness of all three lineages, which showed low identity values.

TAXONOMY

Mycosphaerellaceae* gen*. 1 Nogueira P.T.S. & Pereira O.L., ***gen. nov*.** MycoBank MB in processing.

Type species: *Mycosphaerellaceae* sp. *1* Nogueira P.T.S. & Pereira O.L.

Mycelium internal, septate, pigmented hyphae, smooth. *Stromata* amphigenous, mainly hypophyllous, erumpent, pigmented, textura angularis, well-developed. *Conidiophores* sporodochial, densely fasciculate, pigmented, smooth, aseptate occasionally septate, straight, simple. *Conidiogenous cells* integrated, terminal, dark pigmented, smooth to finely verruculose, monoblastic, proliferating percurrently, conidiogenous loci unthickened and not darkened, apical. *Conidia* solitary, fusiform, cylindrical, subclavate, rarely ypsiliform, pigmented, smooth, thickened wall, apex rounded and subulate, base truncate to obconically truncate, irregularly septate, hila unthickened and not darkened.

Mycosphaerellaceae* sp. *1 Nogueira P.T.S. & Pereira O.L., ***sp. nov.*** MycoBank MB in processing. Fig. 2.

Typus: **Brazil**, Minas Gerais, Universidade Federal de Viçosa, Viçosa, from leaf spots on *Samanea inopinata*, Jun. 2023, *P.T.S. Nogueira* (**holotype** VIC 49570, ex-type culture COAD 3861).

Description in vivo (VIC 49570): *Leaf spots* amphigenous, circular, isolated, rarely coalescent, usually with one sporodochia per spot, dark brown, 0.4–0.9 mm diam. *Mycelium* internal, septate, pigmented hyphae, smooth. *Stromata* amphigenous, mainly hypophyllous, erumpent, pale brown to dark brown, textura angularis, well-developed, 100–220 µm diam. *Conidiophores* sporodochial, brown, smooth to finely verruculose, aseptate or septate, straight, simple, 13–47.5 × 5.5–8(–9) µm. *Conidiogenous cells* integrated, terminal, pale brown to brown, smooth sometime verruculose, monoblastic, proliferating percurrently, conidiogenous loci unthickened and not darkened, apical, 2–4 (–5.5) µm diam. *Conidia* solitary, fusiform, cylindrical, subclavate, rarely ypsiliform, dark brown, smooth, thickened wall, apex rounded and subulate, base truncate to obconically truncate, (24.5–)50–100(–112) × (5–)6–8 µm, irregularly septate (1–)5–15(–21), hila unthickened and not darkened, 2–4 (–5.5) µm diam.

Description in vitro (V8; COAD 3861): *Mycelium* septate, pigmented, smooth. *Conidiophores* sporodochial, densely fasciculate, light brown, smooth to finely verruculose, aseptate or septate, straight, simple, 12–28.5 × (3.5–)4.5–6 µm. *Conidiogenous cells* integrated, terminal, hyaline to pale brown, smooth sometime finely verruculose, monoblastic, proliferating percurrently, conidiogenous loci unthickened and not darkened, apical, 1.5–3 (–3.5) µm diam. *Conidia* solitary, fusiform, cylindrical, subclavate, light brown, smooth, thickened wall, apex rounded and subulate, base truncate to obconically truncate 40–100 × (3.5–) 4–4.5 µm, irregularly septate 5–15, hila unthickened and not darkened, 2–3.5 µm diam.

Culture characteristics: Aerial mycelium dense, convex or umbonate, entire margin, velvety, surface greyish sepia, reverse black. Colonies reaching 2–4 mm diam.

Additional material examined: **Brazil**, Minas Gerais, Universidade Federal de Viçosa, Viçosa, from leaf spots on *Samanea inopinata*, Jun. 2023, *P.T.S. Nogueira* (VIC 49571, living culture COAD 3862).

Notes: Despite morphological similarities with the genus *Pseudocercospora*, such as the presence of sporodochia, inconspicuous conidiogenous loci and hila, percurrent proliferation, and pigmented structures (Crous *et al.* 2013), *Mycosphaerellaceae gen. 1* is not phylogenetically closely related. Surprisingly, *Mycosphaerellaceae* sp. *1* is more closely related to *Asperisporium caricae* and *Asperisporium caricicola*, with which it shares few characteristics, the main ones being the formation of sporodochia and pigmented structures (Minnis *et al.* 2011). The differences with *Asperisporium* species are significant, such as the inconspicuous conidiogenous loci and hila (*Mycosphaerellaceae* sp. 1) vs conspicuous conidiogenous loci and hila (*Asperisporium*), smooth conidia (*Mycosphaerellaceae* sp. 1) vs verrucose conidia (*Asperisporium*), conidia scolecosporous (*Mycosphaerellaceae* sp. 1) vs non-scolecosporous (*Asperisporium*), and percurrently (*Mycosphaerellaceae* sp. 1) vs sympodial conidiogenesis (*Asperisporium*).

Mycosphaerellaceae* gen*. 2 Nogueira P.T.S. & Pereira O.L., ***gen. nov.*** MycoBank MB in processing.

Type species: *Mycosphaerellaceae* sp. *2* Nogueira P.T.S. & Pereira O.L.

Mycelium internal and external, septate, hyaline hyphae. *Stromata* present often absent, erumpent, pigmented, textura angularis. *Conidiophores* emerging from upper part of stromata, fasciculate or emerging in laterally from the external hyphae often reduced to conidiogenous cells, pigmented, straight to sinuous. *Conidiogenous cells* integrated, terminal, pigmented, mono- or polyblastic, proliferating sympodially, with conspicuous conidiogenous loci, refractive, coronate, apical and lateral. *Conidia* catenate, in simple or branched chains, hyaline, smooth straight, obclavate to cylindrical, apex obconically truncate or rounded, often with several conidial hila, base truncate or obconically truncate, conidia aseptate or septate, hila thickened to slightly thickened, darkened.

Mycosphaerellaceae* sp. *2 Nogueira P.T.S. & Pereira O.L., ***sp. nov*.** MycoBank MB in processing. Fig. 3.

Typus: **Brazil**, Minas Gerais, Universidade Federal de Viçosa, Viçosa, on *Erythrina speciosa*, Apr. 2024, *P.T.S. Nogueira* (**holotype** VIC 49574, ex-type culture COAD 3865)

Description in vivo (VIC 49574): *Leaf spots* amphigenous, irregular, isolated or coalescent, buff with black margin and centre, 0.5–14 mm diam. *Mycelium* internal and external, septate, hyaline hyphae, smooth. *Stromata* present often absent, hypophyllous, erumpent, pale brown to brown, textura angularis, lacking to well developed, 30–70 µm diam. *Conidiophores* emerging from upper part of stromata in fascicles or laterally from the external hyphae often reduced to conidiogenous cells, pale brown to brown, smooth, straight to sinuous, simple or branched, (2–)10–70(–120) × (2–) 4–6(–8.5) µm. *Conidiogenous cells* integrated, terminal, hyaline to brown, smooth, mono- or polyblastic (1–4 loci), proliferating sympodially, with conspicuous conidiogenous loci, refractive, coronate, apical and lateral, 1–2.5 µm diam. *Conidia* catenate, in simple or branched chains, forming primary and secondary ramoconidia, hyaline, smooth, straight, obclavate to cylindrical, apex obconically truncate or rounded, often with several conidial hila, base truncate or obconically truncate, (10–)20–90(–160) × 5–7.5 µm, 0–5(–8)-septate, hila thickened to slightly thickened, darkened, 1–2.5 µm diam.

Description in vitro (SNA; COAD 3865): *Mycelium* septate, hyaline to brown, smooth. *Clamidospores* brown in mature, thick walled, one or two celled, in chains rarely solitary, intercalary or terminal, guttulate, subglobose to ellipsoidal, sometimes clavate, pyriform or doliiform, 4–15 × 5–12 µm.

Culture characteristics: Aerial mycelium dense, umbonate, lobate edge, convoluted, sectoring, velvety, diffuse vinaceous black pigment, surface variegated with white, greyish sepia, mouse grey and black, reverse black. Colonies reaching 6–11 mm diam.

Additional material examined: **Brazil**, Minas Gerais, Universidade Federal de Viçosa, Viçosa, leaf spots on *Erythrina speciosa*, Apr. 2024, *P.T.S. Nogueira* (VIC 49575, living culture COAD 3866).

Notes: *Mycosphaerellaceae sp. 2* is placed within a clade containing the genera *Acervuloseptoria*, *Cercosporella*, *Neoacervuloseptoria*, *Neocercosporella*, *Neoramulariopsis*, and *Ramulariopsis*. One of the main morphological differences between *Mycosphaerellaceae gen. 2* and other genera is the presence of pigmented conidiophores and conidiogenous cells. Except for *Acervuloseptoria* and *Neocercosporella*, all other genera in this clade had hyaline conidiogenous cells. The formation of acervular conidiomata in *Acervuloseptoria* and *Neoacervuloseptoria* is clearly different from that in *Mycosphaerellaceae gen. 2*, which forms fascicles. The genus *Neocercosporella* is morphologically closest to *Mycosphaerellaceae gen. 2* in the clade, both can form catenulate conidia and have pigmented conidiophores. *Neocercosporella* can present both hyaline and pigmented conidia, whereas *Mycosphaerellaceae gen. 2* only has hyaline conidia. Another difference is the slightly protuberant conidiogenous loci in *Neocercosporella*, which require electron

microscopy to visualize its coronate shape, in *Mycosphaerellaceae gen. 2*, this feature is more evident and can be observed under light microscopy. Lastly, the hila in *Neocercosporella* are generally unthickened, whereas those in *Mycosphaerellaceae gen. 2* are thickened (Yadav *et al.* 2023).

Mycosphaerellaceae* gen. *3 Nogueira P.T.S. & Pereira O.L., ***gen. nov.*** MycoBank MB in processing.

Type species: *Mycosphaerellaceae* sp. *3* Nogueira P.T.S. & Pereira O.L.

Mycelium internal, septate, smooth, hyaline or pigmented hyphae. *Stromata* amphigenous, mainly hypophyllous, erumpent, subhyaline to pigmented, textura angularis. *Conidiophores* sporodochial to fasciculate, hyaline to pigmented, smooth, aseptate or septate, straight to geniculate-sinuous, simple. *Conidiogenous cells* integrated, terminal, hyaline occasionally pigmented, smooth, mono- or poliblastic, proliferating sympodialy, conidiogenous loci unthickened and not darkened, apical. *Conidia* solitary, filiform, cylindrical, sigmoid, or falcate, hyaline, smooth, guttulate, apex rounded, base obconically truncate, septate, hila somewhat thickened and darkened refractive.

Mycosphaerellaceae* sp. *3 Nogueira P.T.S. & Pereira O.L., ***sp. nov.*** MycoBank MB in processing. Fig. 4.

Typus: **Brazil**, Minas Gerais, Universidade Federal de Viçosa, Viçosa, from leaf spots on *Swietenia macophylla*, Jun. 2024, *P.T.S. Nogueira* (**holotype** VIC 49576, ex-type culture COAD 3867).

Description in vivo (VIC 49570): *Leaf spots* amphigenous, circular,with several white to grey *caespituli*, coalescent, zonate, brown with dark brown margin, 6–45 mm diam. *Mycelium* internal, septate, smooth, hyaline to pale brown hyphae. *Stromata* amphigenous, mainly hypophyllous, erumpent, hyaline to amber or olivaceous, textura angularis, well-developed, 30–135 µm diam. *Conidiophores* sporodochial to fasciculate, hyaline occasionaly to amber or olivaceous, smooth, aseptate reduced a conidiogenous cells occasionally septate, straight to geniculate-sinuous, simple, 9–21 × 3–5.5 µm. *Conidiogenous cells* integrated, terminal, hyaline and occasionally amber or olivaceous, smooth, mono- or polyblastic, proliferating sympodialy, conidiogenous loci unthickened and not darkened, apical sometimes lateral, 1–2.5 µm diam. *Conidia* solitary, filiform, cylindrical, sigmoid, or falcate, hyaline, smooth, guttulate, apex rounded, base truncate to obconically truncate, (12.5–)30–140 × 2.5–4.5 µm, (1–) 6–24, regular-septate, hila sometime thickened, darkened, refractive, (1–) 1.5–2.5 (–3) µm diam.

Description in vitro (SNA; COAD 3867): *Mycelium* septate, smooth, hyaline. *Conidiophores* solitary, hyaline, smooth, reduced a conidiogenous cells or septate, straight to geniculate-sinuous, simple, 10–35 × 3.5–5 µm. *Conidiogenous cells* integrated, terminal, hyaline, smooth, mono- or polyblastic, proliferating sympodialy, conidiogenous loci unthickened and not darkened, apical, 1–2.5 µm diam. *Conidia* solitary, filiform, cylindrical, sigmoid, or falcate, hyaline, smooth, apex rounded, base truncate to obconically truncate, 50–175 × 2.5–7.5 µm, 7–31, regular-septate, hila sometime thickened, not darkened, not refractive, 2–2.5 (–3) µm diam. *Secondary*

conidia thallic, acropetal, doliform, subglobose or clavate, hyaline, smooth base and apex rounded or truncate, aseptate or septate, 6.5–13.5 (–17.5) × 4–8 µm.

Culture characteristics: Aerial mycelium dense, umbonate, radially striate with lobate edge, velvety, diffuse bay pigment, surface apricot to white at margin, reverse radially striate, blood colour to peach at margin. Colonies reaching 13–15 mm diam.

Additional material examined: **Brazil**, Minas Gerais, Universidade Federal de Viçosa, Viçosa, from leaf spots on *Swietenia macophylla*, Mar. 2024, *P.T.S. Nogueira* (VIC 49572, living culture COAD 3863; VIC 49573, living culture COAD 3864).

Notes: In phylogenetic analyses, *Mycosphaerellaceae* sp. *3* appeared as a sister species to *Pantospora chromolaenae*, with strong support values and a long branch separating them. Morphologically, the differences are even more pronounced, as *Mycosphaerellaceae* sp. *3* exhibits hyaline conidia with transverse septation, while *P. chromolaenae* is described with pigmented structures and muriform conidia, which are key characteristics of the genus *Pantospora* and *Sirosporium* (Braun *et al.* 2013, Crous *et al.* 2019). However, phylogeny and additional morphological features were overlooked in the description of *P. chromolaenae*. In addition to the features mentioned, Videira *et al.* (2017) described *Pantospora* as having unthickened hila and conidiogenous loci and non-darkened, whereas *P. chromolaenae* was characterized by thickened, darkened, and refractive scars. Furthermore, the genus was not monophyletic, with *P. chromolaenae* and *P. guazumae* residing in different clades. Therefore, we propose that *P. chromolaenae* should be re-evaluated to be included in *Sirosporium* or a new genus should be established to accommodate it. Furthermore, as far as is known, the characteristic of forming secondary conidia by thallic conidiogenesis is unique in the family.

DISCUSSION

Classifying organisms is not an easy task, nor an exact science, concepts evolve and change over time, and this was no different with the family *Mycosphaerellaceae* Lindau (1897). The main concepts used in its classification have been morphological, ecological, and more recently, phylogenetic features. A polyphasic approach is currently preferred, using phylogeny as a foundation while incorporating other concepts to support and substantiate the proposed new taxa (Videira *et al.* 2016, Yadav *et al.* 2023). In the comparisons made in this study, it became evident that the new genera and species are morphologically distinct from other phylogenetically related species, even in the key characteristics previously used to differentiate the genera within the family, such as the presence or absence of pigmentation in conidiogenous structures, arrangement and branching of conidiophores, placement of conidiogenous cells, proliferation, scar type (conidiogenous loci), and conidial formation, shape, and septation (Chupp 1953, Deighton 1967, Ellis 1971, Deighton 1973, Deighton 1976, Deighton 1979, Braun 1995, Crous *et al.* 2000, Crous 2009).

An important and widely used concept in the taxonomy of the family *Mycosphaerellaceae* is the ecological species concept, defined by Van Valen (1976) as "a lineage (or a closely related set of lineages) which occupies an adaptive zone minimally different from that of any other lineage in its range and which evolves separately from all lineages outside its range." In this context, the adaptive zone of the pathogen corresponds to its host (Crous *et al.* 2000, Aptroot 2006). This concept

can be particularly effective in cases of host-specific taxa, helping, in the absence of molecular data, verify whether the host has already been associated with a species similar to the one being proposed.

When investigating *Samanea inopinata*, the host of *Mycosphaerellaceae* sp. *1*, it was observed that this is a large tree native to the Cerrado (Brazilian Savanna), Caatinga (tropical dry forest), and Atlantic Rainforest biomes (Morim, 2025). Although it is economically underutilized, the species has potential as an ornamental plant for human and animal nutrition and timber exploitation (Carvalho, 2006). There are no records of *Mycosphaerellaceae* fungi associated with *S. inopinata* in the USDA Fungus-Host Databases (https://fungi.ars.usda.gov/) or in '*Mycosphaerella* and allied anamorphs: 1. Names published in *Cercospora* and *Passalora'* (Crous & Braun 2003). The only similar record is that of *Pseudocercospora samaneae* on *Samanea saman*, which can be differentiated from *Mycosphaerellaceae* sp. *1* by the absence of leaf spots, abundant sporulation over the entire leaf surface, and the production of small or absent stromata (Chupp, 1953).

Erythrina speciosa, on the other hand, is a medium-sized tree widely used as an ornamental plant with therapeutic potential, found in the Cerrado and Atlantic Rainforest biomes (Mendonça & Anjos 2006, Fahmy *et al.* 2020, Martins 2025). No *Mycosphaerellaceae* fungi have been reported on *E. speciosa*. However, several records exist for fungi of this family on other species of the genus *Erythrina*, including *Cercospora* (106 records), *Pseudocercospora* (31), *Mycosphaerella* (15), *Septoria* (9), *Exosporium* (7), *Passalora* (3), *Ramularia* (3), and *Cercosporella* (2). Most records can be disregarded due to morphological differences from *Mycosphaerellaceae* sp. *2* or because they have not been identified at the species level. The species most morphologically similar belong to the genus *Cercospora*. However, *C. canescens*, *C erythrinae-lithospermae*, and *C. erythrinicola* belong to the *C. apii* s. lat. complex, distinguishing them from *Mycosphaerellaceae* sp. *2* (Chupp 1953, Crous & Braun 2003).

Finally, *Swietenia macrophylla* is a large tree species found in the Atlantic Forest and Amazon biomes. Heavily exploited by the timber industry, the species is also used as an ornamental plant, but its intensive exploitation for its valuable wood has led to its classification as Vulnerable to extinction on conservation lists (Flores, 2025). Within the genus *Swietenia*, *Pseudocercospora subsessilis* and *P. swieteniae* have been reported, both of which are morphologically distinct from *Mycosphaerellaceae* sp. *3*, which differs by producing hyaline spores (Braun & Urtiaga 2008).

It is worth noting that ecological and morphological species concepts often diverge when compared to the phylogenetic concept. The ecological concept, for example, is rarely applicable to pathogens with a wide host range, while the morphological concept tends to underestimate species diversity, as it cannot differentiate cryptic taxa (Crous *et al.* 2008, Quaedvlieg *et al.* 2014). Although phylogeny is highly efficient, it did not immediately resolve the taxonomic challenges within the family *Mycosphaerellaceae*. Numerous studies over time were required, evolving from single-gene phylogenies to multi-locus analyses, and, in some cases, even incorporating genomic data to understand specific dynamics (Crous *et al.* 1999b, Chang *et al.* 2016, Videira *et al.* 2017). In our concatenated phylogenetic analyses, *Mycosphaerellaceae* gen. *1*, *Mycosphaerellaceae* gen. *2*, and *Mycosphaerellaceae* gen. *3* formed distinct lineages other genera, leading us to propose these new taxa. For *Mycosphaerellaceae* gen. *1* and *Mycosphaerellaceae gen. 3*, all individual analyses formed branches separating them from other genera.

The only exception was *Mycosphaerellaceae* gen*. 2*, which formed this branch only in the *RPB2* phylogeny. However, this result is not uncommon, as other paraphyletic relationships were observed in other genera in the ITS and LSU phylogenies. This phenomenon occurs because *RPB2* is the most informative gene, as evidenced by comparing the number of parsimony-informative loci in this region (532 bp) with ITS (237 bp) and LSU (170 bp). This high informativeness of *RPB2* had already been observed and was one of the main factors justifying its selection as one of the genes used in studies of the family (Videira *et al.* 2016, Videira *et al* 2017, Yadav *et al.* 2023).

Through a polyphasic approach, six new taxa were identified: the genera *Mycosphaerellaceae* gen*. 1*, *Mycosphaerellaceae* gen*. 2*, and *Mycosphaerellaceae* gen*. 3*, and the species *Mycosphaerellaceae* sp. *1*, *Mycosphaerellaceae* sp. *2*, and *Mycosphaerellaceae* sp. *3*. This study is highly relevant as it significantly contributes to fungal diversity estimates at both global and national levels, with a focus on the Atlantic Forest. Although this biome is a well-known biodiversity hotspot, it remains underexplored concerning fungi of the family *Mycosphaerellaceae*, highlighting the need to deepen our understanding of this group. Furthermore, the study of these fungi is essential, considering that cercosporoid fungi are recognized as important plant pathogens. Identifying the etiological agent is the first step in assessing the need for potential interventions, especially in the context of disease management and biodiversity conservation.

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FIGURE AND TABLE LEGENDS

Table 1. Details of genus in *Mycosphaerellaceae* family and sequences used in the phylogenetic analyses. ex-type, ex-epitype or ex-neotype strains are indicated with (T). The species and sequences obtained in this study are highlighted in bold.

Fig. 1. Maximum likelihood phylogenetic tree inferred from the concatenated alignment of ITS, LSU, and *RPB2* sequences using IQ-TREE. Isolates from this study

are highlighted in bold, and type isolates are indicated with "^{T}". Bootstrap (BS) values ≥ 75% and Baysian posterior probabilityn (BPP) values ≥ 0.90 are shown at the nodes ("–" indicates no significant statistical support). Branches with full support (BS = 100% and BPP = 1) are thickened. The tree was rooted with *Schizothyrium pomi* CBS 486.50.

Fig. 2. *Mycosphaerellaceae* sp. *1* COAD 3861, ex-type. **A.** *Samanea tubulosa* showing leaf spots in field. **B.** Leaf spots on upper and lower leaf surface. **C**. Close-up of lesion. **D.** Colony on V8. **E–H.** Fascicles with conidiophores and conidiogenous cells giving rise to conidia. **I.** Condia. **J.** Conidia on V8 culture medium. Scale bars: E = 50 µm; F = 20 µm; G–J = 10 µm.

Fig. 3. *Mycosphaerellaceae* sp. *2* COAD 3865, ex-type. **A.** *Erythrina speciosa* tree on field. **B.** Leaf spots on upper and lower leaf surface. **C.** Close-up of lesion. **D.** Colony on MEA. **E–H.** Fascicles with conidiophores and conidiogenous cells giving rise to conidia. **I**, **J.** Conidia. **K.** Clamidospores on SNA culture medium. Scale bars: E, J = 10 µm; F, G, H, I, K = 20 µm.

Fig. 4. *Mycosphaerellaceae* sp. *3* COAD 3867, ex-type. **A.** Leaf spots on upper and lower leaf surface of *Swietenia macophylla*. **B.** Close-up of lesion. **C.** Colony on MEA. **D.** Colony surface on SNA. **E–G.** Fascicles with conidiophores and conidiogenous cells giving rise to conidia. **H.** Conidia. **I–J.** Conidiogenous cell and conidia on SNA. **K.** Secondary conidia on SNA. Scale bars: E = 50 µm; F, G, K = 20 µm; I, J = 10 µm.

Supplementary Fig. S1. Maximum likelihood phylogenetic tree inferred from the individual alignment of ITS sequence using IQ-TREE. Isolates from this study are highlighted in bold, and type isolates are indicated with "T". Bootstrap (BS) values ≥ 75% are shown at the nodes. The tree was rooted with *Schizothyrium pomi* CBS 486.50.

Supplementary Fig. S2. Maximum likelihood phylogenetic tree inferred from the individual alignment of LSU sequence using IQ-TREE. Isolates from this study are highlighted in bold, and type isolates are indicated with "T". Bootstrap (BS) values ≥ 75% are shown at the nodes. The tree was rooted with *Schizothyrium pomi* CBS 486.50.

Supplementary Fig. S3. Maximum likelihood phylogenetic tree inferred from the individual alignment of *RPB2* sequence using IQ-TREE. Isolates from this study are highlighted in bold, and type isolates are indicated with "T". Bootstrap (BS) values ≥ 75% are shown at the nodes. The tree was rooted with *Schizothyrium pomi* CBS 486.50.

TABLE AND FIGURES

Table 1.

Taxa	Host/Substrate	Strains	GenBank accession numbers			References
			LSU	ITS	*RPB2*	
Acericercospora hyrcanica	*Acer cappadocicum*	IRAN 4555C (T)	ON226842	ON212664	–	Bakhshi and Braun 2021
Acervuloseptoria ziziphicola	*Ziziphus mucronata*	CBS 138009 = CPC 23707 (T)	KJ869221	KJ869164	MF951425	Videira et al. 2017
Amycosphaerella africana	*Eucalyptus viminalis*	CBS 680.95 = CPC 796 (T)	KF902048	KF901701	MF951426	Videira et al. 2017
Amycosphaerella keniensis	*Eucalyptus grandis* litter	CBS 111001 = CPC 1084 = CMW 5147 (T)	GQ852610	MF951290	MF951433	Videira et al. 2017
Annellosympodiella juniperi	*Juniperus procera*	CBS 137992 = CPC 23276 (T)	KJ869204	NR156284	MF951436	Videira et al. 2017
Apseudocercosporella trigonotidis	*Trigonotis peduncularis*	CBS 131890 = CPC 10864 (T)	JQ324972	GU269858	KX288414	Videira et al. 2017
Asperisporium caricae	*Carica papaya*	CBS 130298 (T)	MF951128	JN190955	MF951437	Videira et al. 2017
Asperisporium caricicola	*Carica papaya*	CBS 139998 = CPC 24348 = TSU:MUMH (T)	KR611891	KR611869	MF951439	Videira et al. 2017

Mycosphaerellaceae* sp. *1	***Samanea inopinata***	**COAD 3861 (T)**	**in process**	**in process**	**in process**	This study
Mycosphaerellaceae* sp. *1	***Samanea inopinata***	**COAD 3862**	**in process**	**in process**	**in process**	This study
Australosphaerella nootherensis	*Corymbia intermedia*	CBS 130522 (T)	KF901835	MF951293	MF951440	Videira et al. 2017
Brunneosphaerella protearum	*Protea* sp.	CBS 130597 = CPC 16338 (T)	GU214397	GU214626	MF951443	Videira et al. 2017
Brunswikiella parsonsiae	*Parsonsia straminea*	CBS 137979 = CPC 22537 (T)	KJ869188	KJ869131	MF951593	Videira et al. 2017
Caryophilloseptoria pseudolychnidis	*Lychnis cognata*	CBS 128630 (T)	KF251795	KF251291	MF951446	Videira et al. 2017
Mycosphaerellaceae* sp. *2	***Erythrina speciosa***	**COAD 3865 (T)**	**in process**	**in process**	**in process**	This study
Mycosphaerellaceae* sp. *2	***Erythrina speciosa***	**COAD 3866**	**in process**	**in process**	**in process**	This study
Catenulocercospora fusimaculans	*Agrostis* sp.	CPC 17277	KF251817	KF251313	MF951450	Videira et al. 2017
Cercoramularia koreana	*Styrax japonicus*	CBS 142175 = CPC 10709 (T)	MF951132	MF951296	MF951453	Videira et al. 2017
Cercospora armoraciae	*Berteroa incana*	CBS 538.71	MF951134	JX143547	MF951454	Videira et al. 2017
Cercospora zeina	*Zea mays*	CBS 118820 = CPC 11995 (T)	MF951147	DQ185081	MF951469	Videira et al. 2017

Cercosporella virgaureae	*Erigeron annuus*	CPC 11460	KX286976	KX287283	KX288426	Videira et al. 2017
Cercosporidium californicum	*Asclepias fascicularis*	CBS 128857 = CPC 18389 (T)	MF951148	HQ728115	MF951470	Videira et al. 2017
Cercosporidium chaetomium	*Euphorbia* sp.	CBS 142177 = CPC 18624 (T)	MF951151	MF951306	MF951474	Videira et al. 2017
Chuppomyces handelii	*Rhododendron* sp.	CBS 113302	GU214437	EU167581	MF951475	Videira et al. 2017
Cladocillium musae	*Musa itinerana*	BCRC FU30634 (T)	NG074458	NR171262	LC546944	Chen et al. 2020
Clarohilum henningsii	*Manihot esculenta*	CPC 17314	MF951152	MF951307	MF951476	Videira et al. 2017
Clypeosphaerella quasiparki	*Eucalyptus* sp.	CBS 123243 = CPC 15409 (T)	KF902128	KF901771	MF951478	Videira et al. 2017
Collapsimycopappus styracis	*Styrax obassia*	HHUF 30067 (T)	LC333036	LC333030	LC333042	Hashimoto et al. 2018
Collarispora valgourgensis	*Yucca* sp.	CBS 129531 = CPC 18385 (T)	JF951175	JF951152	MF951479	Videira et al. 2017
Coremiopassalora eucalypti	*Eucalyptus saligna*	CBS 111318	GU253860	GU269845	MF951482	Videira et al. 2017
Cytostagonospora martiniana	*Acacia pycnantha*	CBS 135102 = CPC 17727 (T)	KF251657	KF251153	MF951484	Videira et al. 2017
Deightonomyces daleae	*Dalea spinosa*	CBS 113031	MF951155	EU040236	MF951485	Videira et al. 2017

Devonomyces endophyticus	*Eucalyptus* sp.	CBS 114662 (T)	KF902060	KF901713	MF951590	Videira et al. 2017
Dictyosporina ferruginea	*Calophyllaceae*	COAD 2272 (T)	MF344902	MF344901	_	Hyde et al. 2017
Distocercospora pachyderma	*Dioscorea* sp.	CBS 138247 = CPC 24144 (T)	MF951156	MF951311	MF951486	Videira et al. 2017
Distocercosporaster dioscoreae	*Dioscorea tenuipes*	CBS 135463 = CPC 11513	KF251815	KF251311	MF951489	Videira et al. 2017
Distomycovellosiella brachycarpa	*Solanum mauritianum*	CBS 142178 = CPC 18381 (T)	MF951158	MF951313	MF951490	Videira et al. 2017
Dothistroma pini	*Pinus nigra*	CBS 116486	JX901823	JX901735	KX348053	Videira et al. 2017
Epicoleosporium ramularioides	*Coleosporium phellodendri on leaves of Phellodendron amurense*	CPC 10673	MF951160	KX287289	KX288434	Videira et al. 2017
Exosporium livistonae	*Livistona benthamii*	CBS 131313 = CPC 19357 (T)	JQ044446	JQ044427	MF951494	Videira et al. 2017
Exutisphaerella laricina	*Larix decidua*	CBS 326.52 (T)	GU253693	GU269643	MF951496	Videira et al. 2017
Filiella pastinacae	*Laserpitium latifolium*	CBS 114116	KF251832	KF251328	KX348056	Videira et al. 2017
Fulvia fulva	*Solanum lycopersicum*	CBS 142314 = CPC	MF951163	MF951317	MF951498	Videira et al. 2017

		13652 (T)				
Fusoidiella depressa	*Angelica gigas*	CBS 141335 = CPC 14915	KF251813	KF251309	KX348055	Videira et al. 2017
Graminopassalora graminis	*Alopecurus aequalis* var. *amurensis*	CBS 113303	GU214666	GU214666	MF951502	Videira et al. 2017
Hippopotamyces phragmitis	*Phragmites australis*	CBS 146086 = CPC 36385	MN567630	MN562122	MN556803	Crous et al 2019b
Hirudinaria macrocarpa	*Crataegus* sp.	CBS 150858 (T)	OR785993	OR785997	–	Bakhshi and Crous 2024
Hirudinaria mespili	*Mespilus germanica*	CBS 150859 (T)	OR785996	OR786000	OR790975	Bakhshi and Crous 2024
Hyalocercosporidium desmodii	*Desmodium tortuosum*	CBS 142179 = CPC 19483 (T)	MF951168	MF951322	MF951503	Videira et al. 2017
Hyalozasmidium aerohyalinosporum	*Eucalyptus tectifica*	CBS 125011 (T)	KF901930	GQ852839	MF951504	Videira et al. 2017
Juncomyces californiensis	*Juncus effusus*	CBS 146631 = CPC 37993 (T)	MT373352	MT373369	MT375101	Crous et al. 2020
Lecanosticta acicola	*Pinus strobus*	CBS 133791 (T)	KC013017	KC012999	MF951507	Videira et al. 2017
Madagascaromyces intermedius	*Eucalyptus camaldulensis*	CBS 124154 (T)	FJ790297	FJ790267	MF951511	Videira et al. 2017
Microcyclosporella mali	*Malus domestica*	CBS 126136 = CPC 16184 (T)	GU570547	GU570535	KX288436	Videira et al. 2017

Micronematomyces caribensis	*Chromolaena odorata*	CBS 126136 = CPC 16184 (T)	MF951175	DQ676515	MF951517	Videira et al. 2017
Micronematomyces chromolaenae	*Chromolaena odorata*	CBS 113611 = MJM 1498 = C452 (T)	MF951180	DQ676518	MF951522	Videira et al. 2017
Miuraea degenerans	*Prunus mume*	MAFF 239265 = MUCC 1514 (T)	MF951181	MF951325	MF951523	Videira et al. 2017
Mycodiella sumatrensis	*Eucalyptus* sp.	CBS 118501	JX901872	DQ303049	MF951525	Videira et al. 2017
Mycosphaerelloides madeirae	*Eucalyptus globulus*	CBS 112895 = CPC 3745 (T)	KF902017	AY725553	KX348057	Videira et al. 2017
Mycovellosiella cajani	*Cajanus cajan*	CBS 113998	KF251819	KF251315	MF951527	Videira et al. 2017
Neoacervuloseptoria fraxini	*Fraxinus* sp.	CPC 36558 (T)	NG073868	NR170046	MT223673	Yadav et al. 2023
Neoceratosperma eucalypti	*Eucalyptus* sp.	CBS 137998 = CPC 23465 (T)	KJ869210	KJ869153	MF951531	Videira et al. 2017
Neocercospora ammicola	*Ammi majus*	CBS 136450 (T)	KR232405	KR232407	KX288446	Videira et al. 2017
Neocercosporella peristrophes	*Peristrophe bicalyculata*	AMH 9671 (T)	MZ311874	MZ311866	OL773683	Yadav et al. 2023
Neocercosporidium smilacis	*Smilax aspera*	CBS 122888 (T)	MF951185	MF951329	MF951536	Videira et al. 2017
Neodeightoniella phragmiticola	*Phragmites australis*	CBS 136418 = CPC	KF777224	KF777171	MF951543	Videira et al. 2017

		22059 (T)				
Neokamalomyces indicus	*Ficus benghalensis.*	NFCCI 4870	NG228855	NR185416	OL773682	Yadav et al. 2022
Neokirramyces syzygii	*Syzygium* sp.	CBS 146050 =CPC 36122 (T)	MN567623	MN562115	–	Crous et al 2019b
Neomycosphaerella pseudopentameridis	*Pseudopentameris macrantha*	CBS 136407 = CPC 21126 (T)	KF777226	KF777173	MF951545	Videira et al. 2017
Neopenidiella nectandrae	*Nectandra coriacea*	CBS 734.87 (T)	KF901982	MF951335	MF951546	Videira et al. 2017
Neophloeospora maculans	*Morus alba*	CBS 115123	GU214670	GU214670	MF951547	Videira et al. 2017
Neopseudocercosporella capsellae	*Brassica* sp.	CBS 112032	KF251824	KF251320	KX348060	Videira et al. 2017
Neoramulariopsis catenulata	*Phaseolus vulgaris*	CBS 355.73 (T)	KX286973	KX287281	KX288424	Videira et al. 2017
Neoramulariopsis unguis-cati	*Dolichandra unguiscati*	CBS 138101 = CPC 22948 (T)	KJ869197	KJ869140	KX288423	Yadav et al. 2023
Neoramichloridium bambusicola	*Bambusa* sp.	MFLUCC 15–0455 (T)	KY205720	KY205719	–	Thambugala et al 2017
Neoseptoria caricis	*Carex acutiformis*	CBS 135097 (T)	KF251663	KF251159	MF951551	Videira et al. 2017
Neosonderhenia eucalypti	*Eucalyptus costata*	CBS 145081 = CPC 34405	NG067911	NR165602	MN162578	Crous et al. 2019c
Nothopassalora personata	*Arachis hypogaea*	CBS 142236 = CPC	MF951235	MF951374	MF951632	Videira et al. 2017

		19466 (T)				
Nothopericoniella perseae-macranthae	*Machilus zihoensis*	CBS 122097	GU452682	MF951354	MF951583	Videira et al. 2017
Nothophaeocryptopus gaeomannii	–	CBS 244.38	MF951191	MF951336	GU371740	Videira et al. 2017
Nothopseudocercospora dictamni	*Dictamnus albus*	CPC 39776 = CBS 148299 (T)	ON811571	ON811513	ON803549	Crous et al. 2022
Nothoseptoria caraganae	*Caragana arborescens*	CPC 36563 = CBS 145993 (T)	MT223917	MT223825	MT223693	Crous et al. 2020
Pachyramichloridium pini	*Pinus contorta*	CBS 461.82 (T)	EU041859	EU041802	MF951552	Videira et al. 2017
Pallidocercospora heimii	*Eucalyptus sp.*	CBS 110682 = CPC 760 (T)	GQ852604	KF901671	MF951554	Videira et al. 2017
Pantospora chromolaenae	*Chromolaena odorata*	CBS 145563	MK876442	NR165571	MK876488	Crous et al. 2019a
Pantospora guazumae	*Guazuma ulmifolia*	CBS 130299 (T)	MF951196	JN190956	MF951556	Videira et al. 2017
Paracercospora egenula	*Solanum melongena*	CBS 132030	GU253738	GU269698	MF951557	Videira et al. 2017
Paracercosporidium microsorum	*Tilia cordata*	CBS 142176 = CPC 15550 (T)	MF951201	MF951344	MF951564	Videira et al. 2017
Paracercosporidium tiliae	*Tilia americana*	CBS 112734 = CPC 3952 (T)	MF951202	MF951345	MF951565	Videira et al. 2017

Paramycosphaerella brachystegia	*Brachystegia sp.*	CBS 136436 = CPC 21136 (T)	KF777230	KF777178	MF951567	Videira et al. 2017
Paramycovellosiella passaloroides	*Amorpha fruticosa*	CPC 10770	MF951209	MF951352	MF951580	Videira et al. 2017
Parapallidocercospora colombi-ensis	*Eucalyptus urophylla*	CBS 110968 = CPC 1105 (T)	KF901969	AY752148	MF951581	Videira et al. 2017
Passalora bacilligera	*Alnus glutinosa*	CBS 131547 (T)	MF951210	MF951356	MF951585	Videira et al. 2017
Passalora vaginae	*Saccharum officinarum*	CBS 140.34	MF951166	MF951320	_	Videira et al. 2017
Pedrocrousiella pongamiae	*Pongamia pinnata*	NFCCI 4881 (T)	MW327593	MW327548	MW363496	Rajeshkumar et al. 2021
Phaeocercospora colophospermi	*Colophospermum mopane*	CBS 132687 = CPC 19812 (T)	JX069854	JX069870	MF951586	Videira et al. 2017
Phaeophleospora eugeniae	*Eugenia uniflora*	CBS 142184 = CPC 15143	FJ493206	FJ493188	MF951594	Videira et al. 2017
Phaeoramularia gomphrenicola	*Pfaffia glomerata*	CBS 142182 = CPC 23248 (T)	MF951216	MF951359	MF951599	Videira et al. 2017
Phloeospora ulmi	*Ulmus sp.*	CBS 101564	KF251703	KF251200	MF951602	Videira et al. 2017
Pleopassalora perplexa	*Acacia crassicarpa*	CBS 116363 = CPC 11147 (T)	MF951220	AY752162	MF951606	Videira et al. 2017

Pleuropassalora armatae	*Dalbergia armata*	CBS 125420 = CPC 15419 (T)	GU214456	GU214640	MF951609	Videira et al. 2017
Pleurovularia polliniae	*Pollinia imberbis*	KUS-F33118	OR573653	OR573650	OR575844	Choi et al. 2024
Pluripassalora bougainvilleae	*Bougainvillea sp.*	CBS 142237 = CPC 19327	MF951224	MF951365	MF951612	Videira et al. 2017
Plurivorosphaerella nawae	*Diospyros* sp.	MN6-1	LC380932	LC194865	LC380941	Hassan & Chang 2018
Polyphialoseptoria terminaliae	*Terminalia catappa*	CBS 135106 = CPC 19611 (T)	KF251717	KF251214	MF951615	Videira et al. 2017
Prathigadoides gleditsiae-caspicae	*Gleditsia caspica*	CBS 136121 (T)	MZ423829	MZ423851	MZ427652	Bakhshi et. al 2021
Protostegia eucleae	*Euclea undulata*	CPC 23549 = CBS 137232 (T)	KR873280	KR873252	–	Crous et al. 2015
Pruniphilomyces circumscissus	*Prunus cerasus*	CPC 36434 = CBS 145985	MT223926	MT223834	MT223697	Crous et al. 2020
Pseudocercospora vitis	*Vitis vinifera*	CBS 132012	GU214483	GU269829	KX348076	Videira et al. 2017
Pseudocercospora zambiae	*Terminalia sp.*	CBS 136423 = CPC 22685 (T)	KF777228	KF777175	MF951630	Videira et al. 2017
Pseudocercosporella bakeri	*Ipomoea aquatica*	CBS 125685 = CPC 17570 (T)	KX287005	KX287306	KX288462	Videira et al. 2017

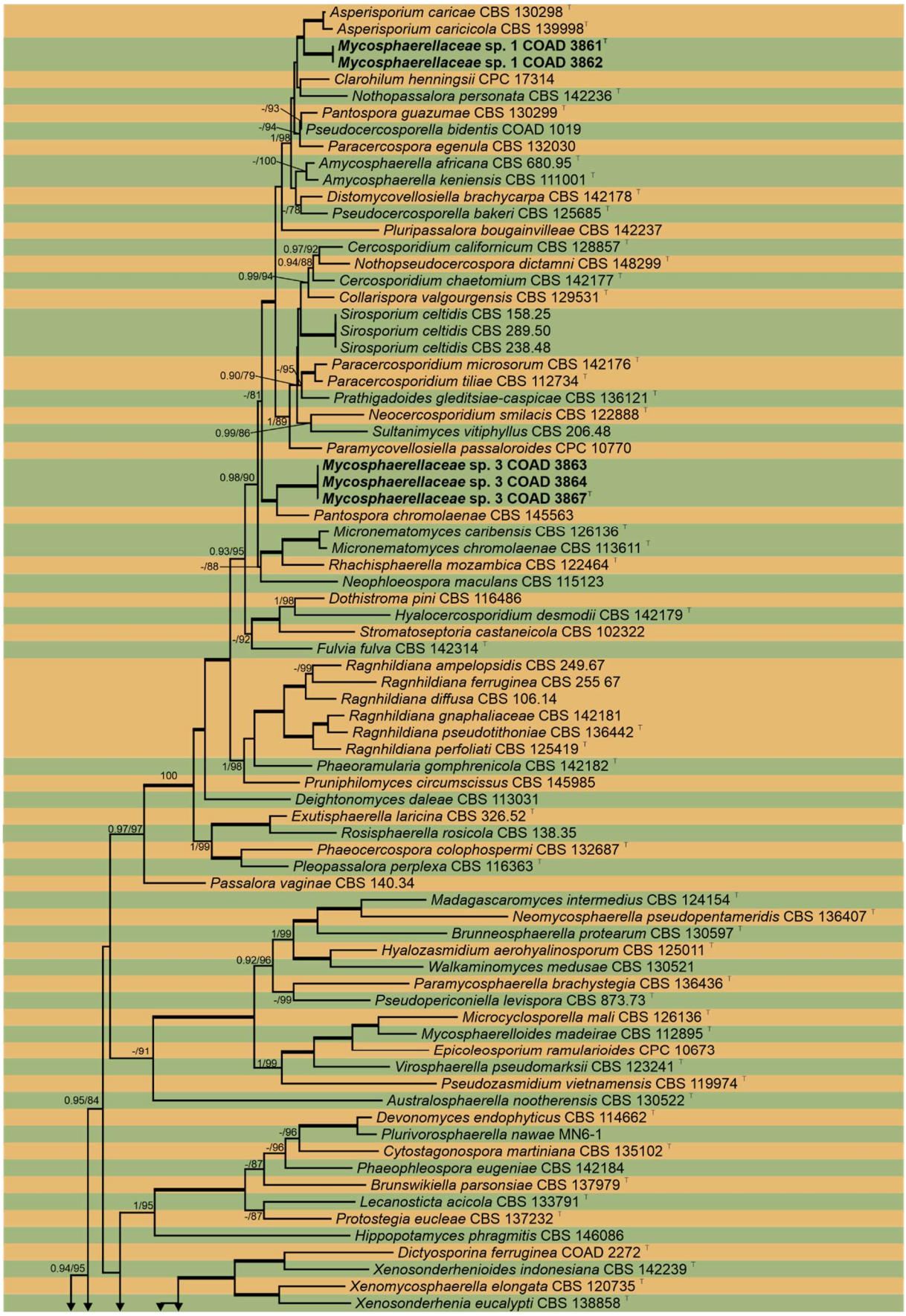
Pseudocercosporella bidentis	*Bidens subalternans*	COAD 1019	KF421120	KF421114	–	Guatimosim et al. 2015
Pseudopericoniella levispora	*Turpinia pomifera*	CBS 873.73 (T)	EU041837	EU041780	MF951633	Videira et al. 2017
Pseudophaeophleospora stonei	*Eucalyptus* sp.	CBS 120830 = CPC 13330 (T)	FJ493210	EF394856	MF951636	Videira et al. 2017
Pseudozasmidium vietnamensis	*Eucalyptus grandis*	CBS 119974 (T)	JF700944	DQ632675	MF951639	Videira et al. 2017
Pteridopassalora lygodii	*Lygodium japonicum*	BCRC FU30503 (T)	–	KR527201	–	Kirschner & Wang (2015)
Pteridopassalora nephrolepidicol	*Nephrolepis falcata*	CBS 128211 (T)	HQ599591	HQ599590	KX462646	Chen et al. 2022
Ragnhildiana ampelopsidis	*Parthenocissus tricuspidata*	CBS 249.67 = IMI 124968	MF951238	AY293063	MF951641	Videira et al. 2017
Ragnhildiana diffusa	*Carya illinoinensis*	CBS 106.14	MF951239	MF951375	MF951642	Videira et al. 2017
Ragnhildiana ferruginea	*Artemisia vulgaris*	CBS 255.67 = IMI 124973	MF951241	MF951377	MF951644	Videira et al. 2017
Ragnhildiana gnaphaliaceae	*Gnaphalium affine*	CBS 142181 = CPC 12517	MF951243	MF951379	MF951646	Videira et al. 2017
Ragnhildiana perfoliati	*Ageratina adenophora*	CBS 125419 = CPC 15365 (T)	GU214453	GU214639	MF951647	Videira et al. 2017
Ragnhildiana pseudotithoniae	*Tithonia diversifolia*	CBS 136442 = CPC 21688 (T)	KF777231	KF777179	MF951652	Videira et al. 2017

Ramularia pusilla	*Poa annua*	CBS 124973 (T)	KP894141	KP894248	KP894687	Videira et al. 2017
Ramulariopsis gossypii	*Gossypium* sp.	CBS 141099 (T)	KX287243	KX287540	KX288702	Videira et al. 2017
Ramulariopsis pseudoglycines	*Gossypium* sp.	CBS 141100 (T)	KX287246	KX287543	KX288705	Videira et al. 2017
Ramulispora sorghi	*Sorghum bicolor*	CBS 111032	MF951248	MF951384	MF951654	Videira et al. 2017
Rhachisphaerella mozambica	*Musa acuminata*	CBS 122464 (T)	MF951237	EU514257	MF951640	Videira et al. 2017
Rosisphaerella rosicola	–	CBS 138.35	MF951252	MF951388	MF951658	Videira et al. 2017
Ruptoseptoria unedonis	*Arbutus unedo*	CBS 755.70	KF251732	KF251229	MF951659	Videira et al. 2017
Schizothyrium pomi	*Polygonum sachalinense*	CBS 486.50	EF134948	EF134948	MF951735	Videira et al. 2017
Scolecostigmina mangiferae	*Mangifera indica*	CBS 125467 = CPC 17351 (T)	GU253877	GU269870	MF951660	Videira et al. 2017
Septoria proteacearum	*Zantedeschia aethiopica*	CBS 135477 = CPC 19675	KF252029	KF251524	MF951663	Videira et al. 2017
Sirosporium celtidis	*Celtis australis*	CBS 158.25	MF951253	MF951389	MF951669	Videira et al. 2017
Sirosporium celtidis	–	CBS 238.48	MF951254	MF951390	MF951670	Videira et al. 2017
Sirosporium celtidis	*Celtis australis*	CBS 289.50	MF951255	MF951391	MF951671	Videira et al. 2017

Sonderhenia eucalyptorum	*Eucalyptus coccifera*	CBS 120220	DQ923536	DQ923536	MF951673	Videira et al. 2017
Sphaerulina tirolensis	*Rubus idaeus*	CBS 109018 (T)	KF252143	KF251638	MF951680	Videira et al. 2017
Stromatoseptoria castaneicola	*Castanea sativa*	CBS 102322	KF251774	KF251271	MF951681	Videira et al. 2017
Sultanimyces vitiphyllus	*Vitis* sp.	CBS 206.48	MF951260	MF951395	MF951683	Videira et al. 2017
Mycosphaerellaceae* sp. *3	***Swietenia macophylla***	**COAD 3863**	**in process**	**in process**	**in process**	This study
Mycosphaerellaceae* sp. *3	***Swietenia macophylla***	**COAD 3864**	**in process**	**in process**	**in process**	This study
Mycosphaerellaceae* sp. *3	***Swietenia macophylla***	**COAD 3867 (T)**	**in process**	**in process**	**in process**	This study
Trochophora simplex	*Daphniphyllum macropodum*	CBS 124744	GU253880	GU269872	MF951684	Videira et al. 2017
Uwemyces elaeidis	*Elaeis oleifera*	CPUwZC 01	KX228356	KX228299	KX228371	Videira et al. 2017
Virosphaerella pseudomarksii	*Eucalyptus* sp.	CBS 123241 = CPC 15410 (T)	KF902127	KF901770	MF951686	Videira et al. 2017
Walkaminomyces medusae	*Eucalyptus alba*	BRIP 52586 = CBS 130521	NG067910	NR137044	MN239111	Carnegie et al. 2011
Xenomycosphaerella elongata	*Eucalyptus calmadulensis* × *urophylla*	CBS 120735 = CPC 13378 (T)	JF700942	EF394833	MF951687	Videira et al. 2017

Xenoramularia polygonicola	*Polygonum* sp.	CBS 141102 = CPC 10852 (T)	GU214695	GU214695	KX288723	Videira et al. 2017
Xenosonderhenia eucalypti	*Eucalyptus urophylla*	CBS 138858 = CPC 24247 (T)	KP004485	KP004457	MF951688	Videira et al. 2017
Xenosonderhenioides indonesiana	*Eucalyptus* sp.	CBS 142239 = CPC 15066 (T)	MF951261	MF951396	MF951689	Videira et al. 2017
Zasmidium cellare	Wall in wine cellar	CBS 146.36 (T)	EU041878	EU041821	MF951693	Videira et al. 2017
Zymoseptoria tritici	*Triticum aestivum*	CBS 115943 (T)	GU214436	AF181692	KX348112	Videira et al. 2017

Fig. 1.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Paracercospora egenula CBS 132030
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Collarispora valgourgensis CBS 129531 T
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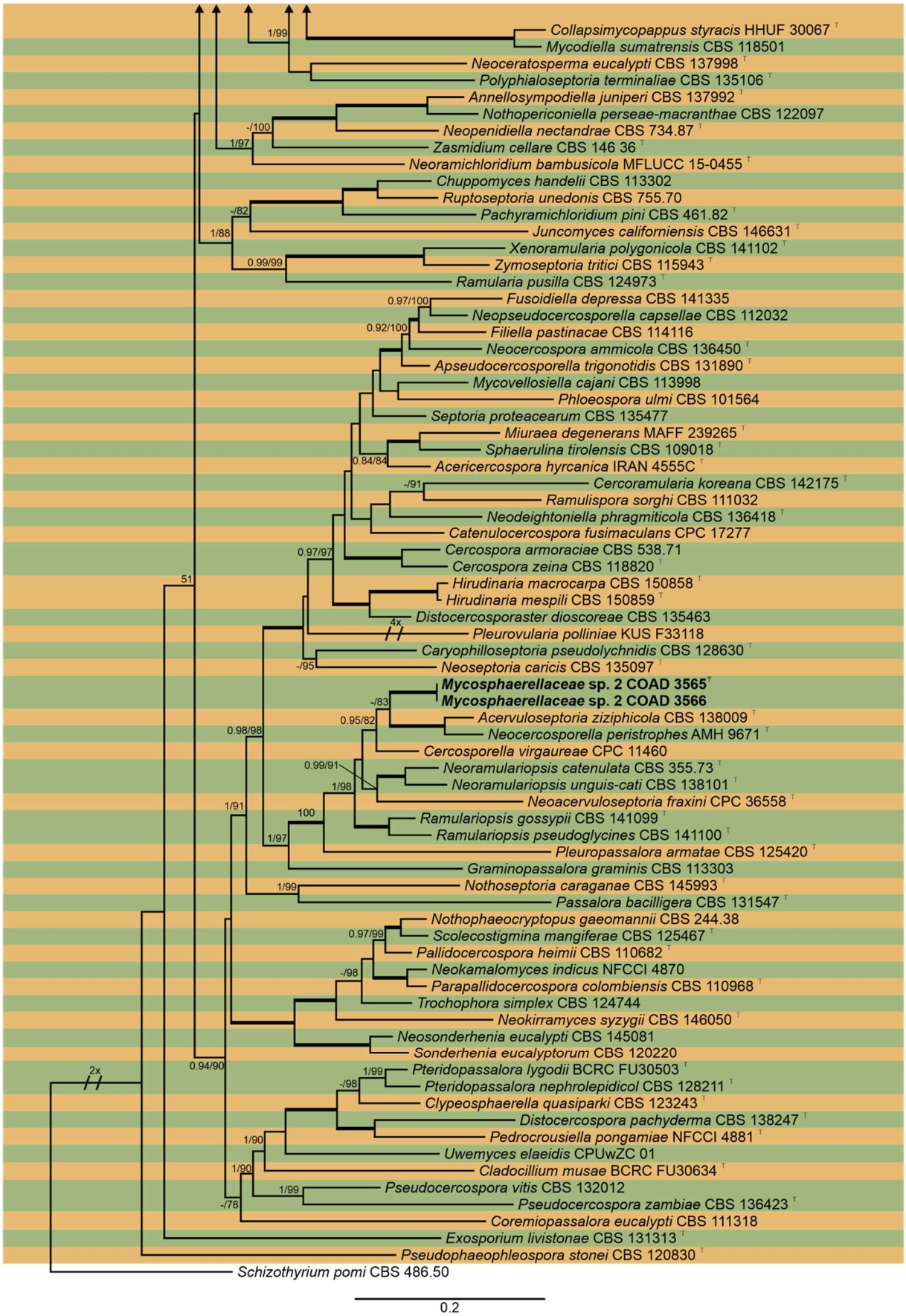
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Fig. 2.

Fig. 3.

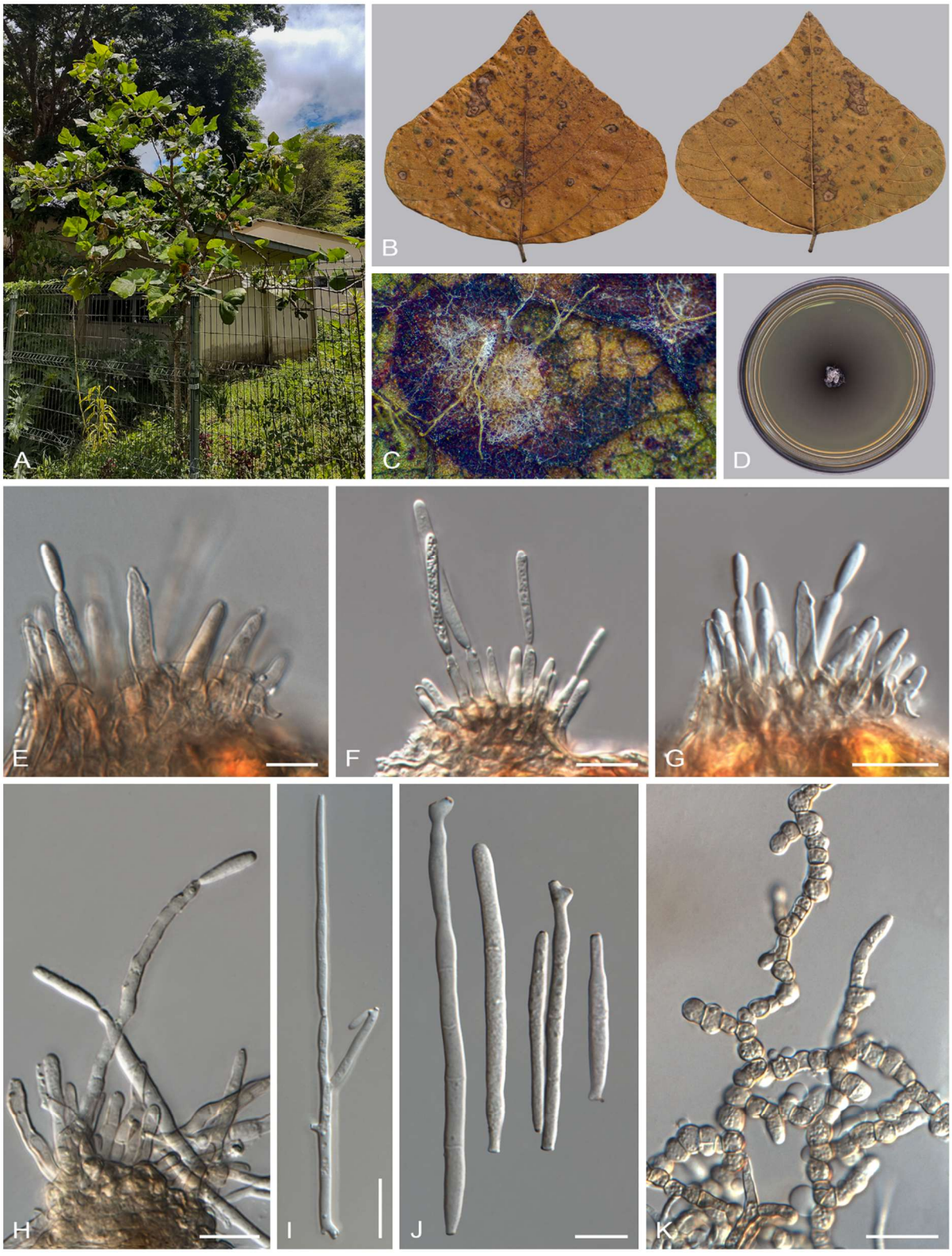
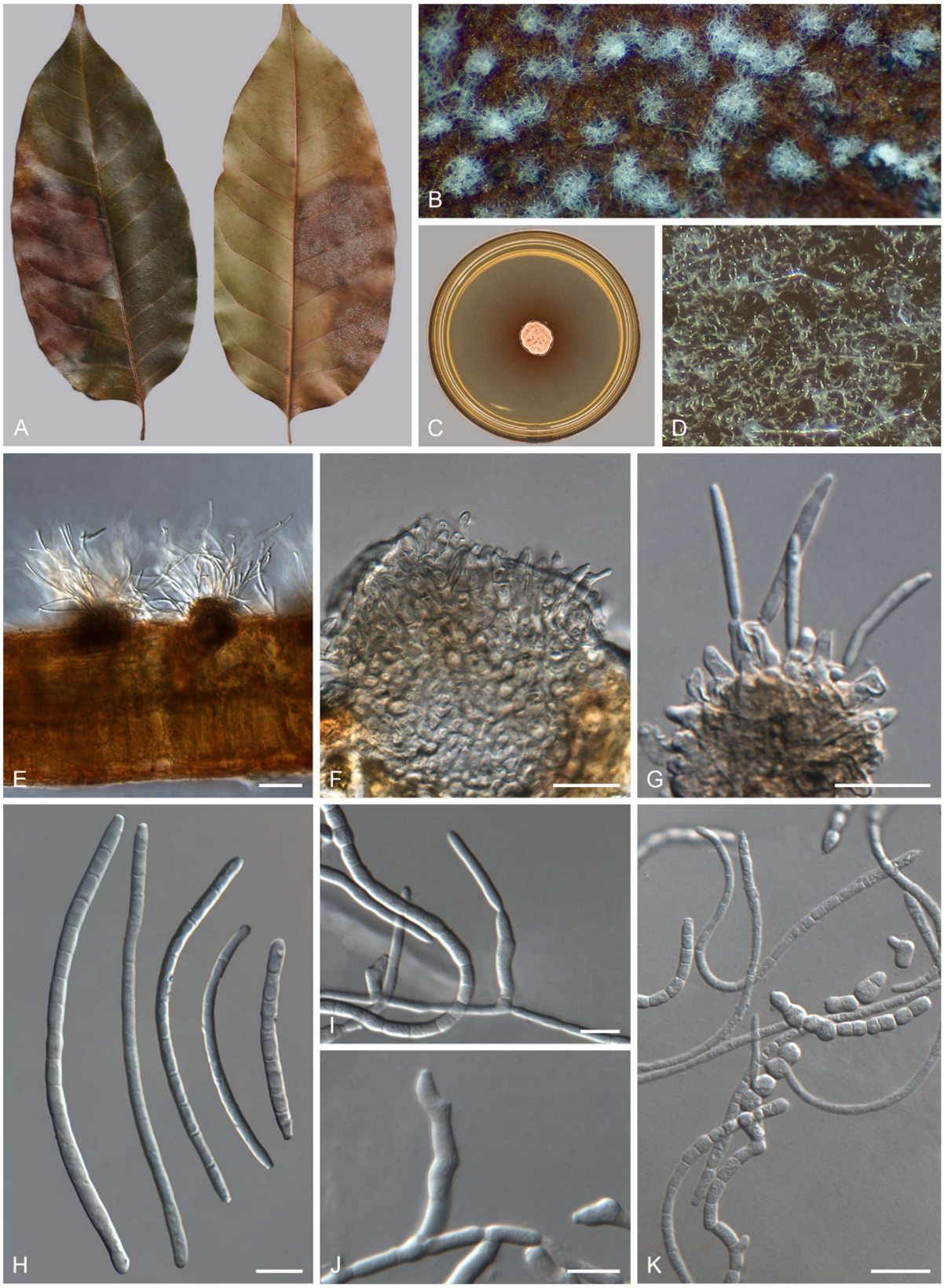
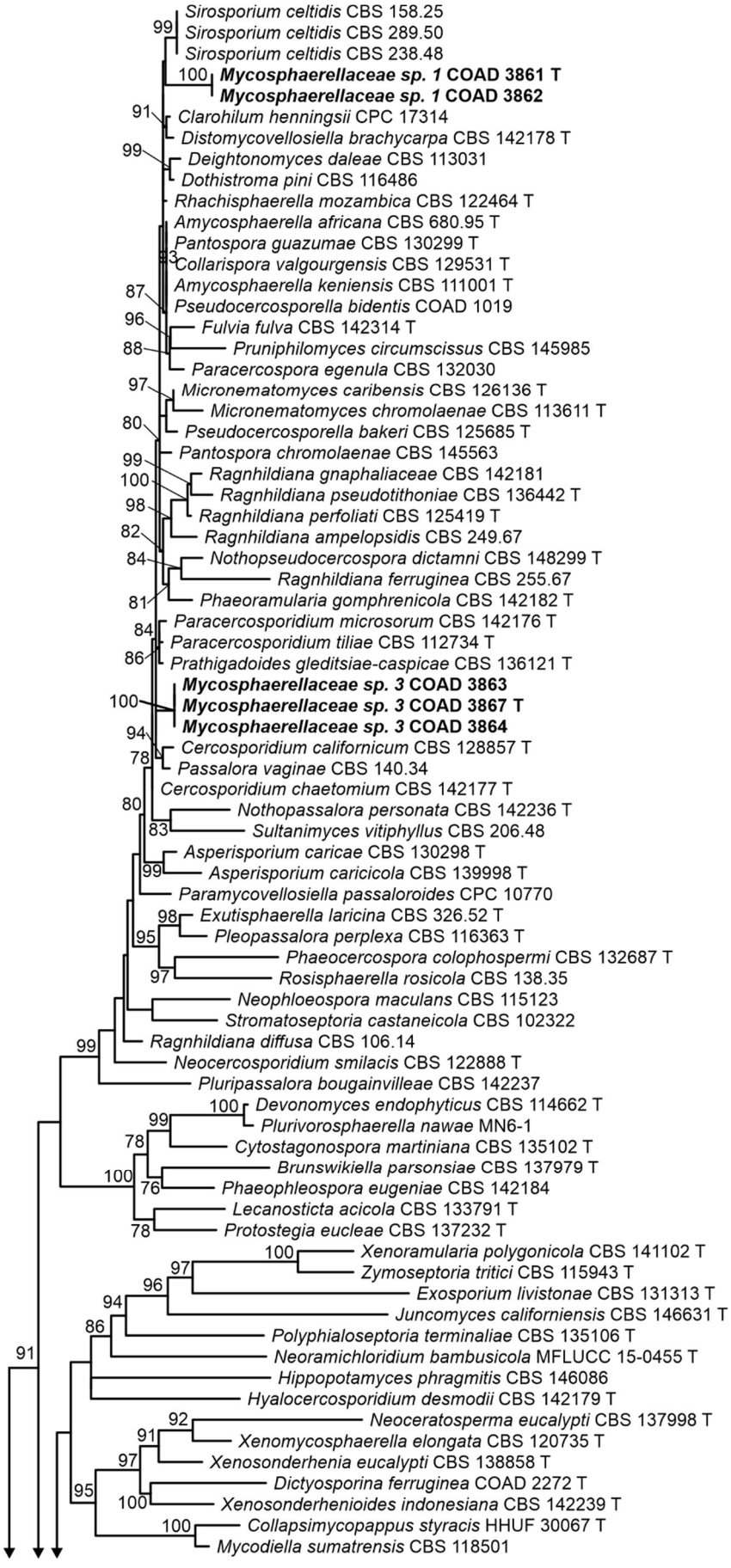
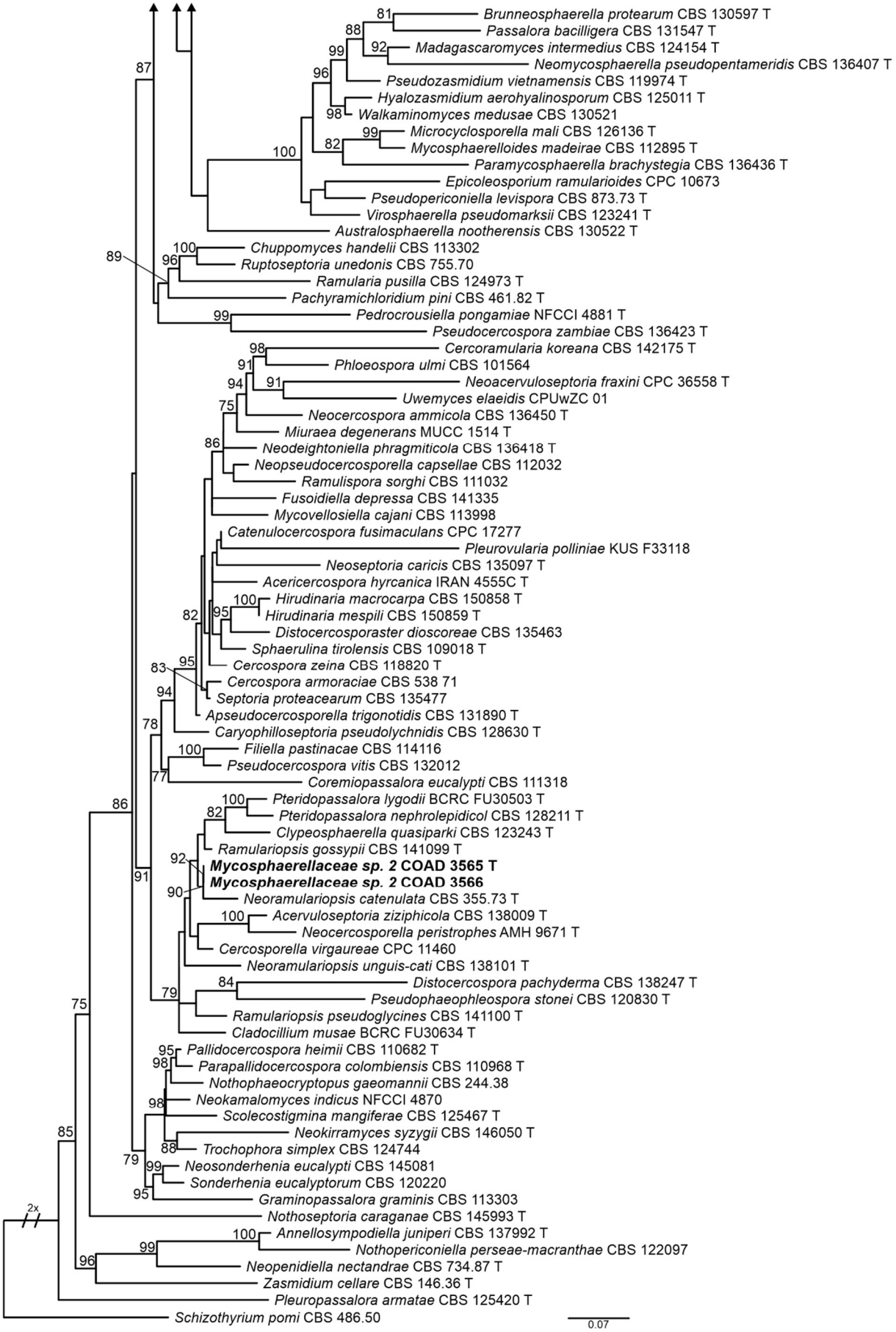


Fig. 4.



Supplementary Fig. S1.

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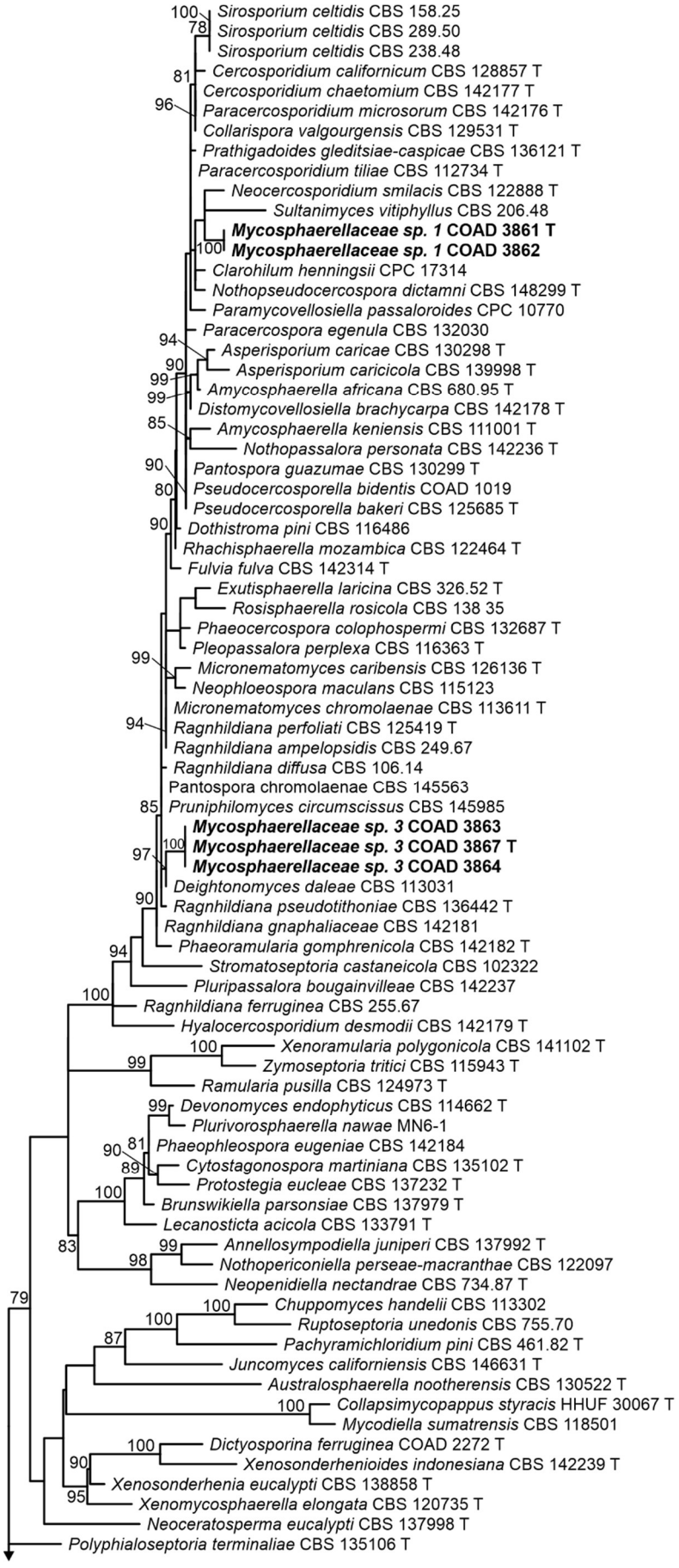
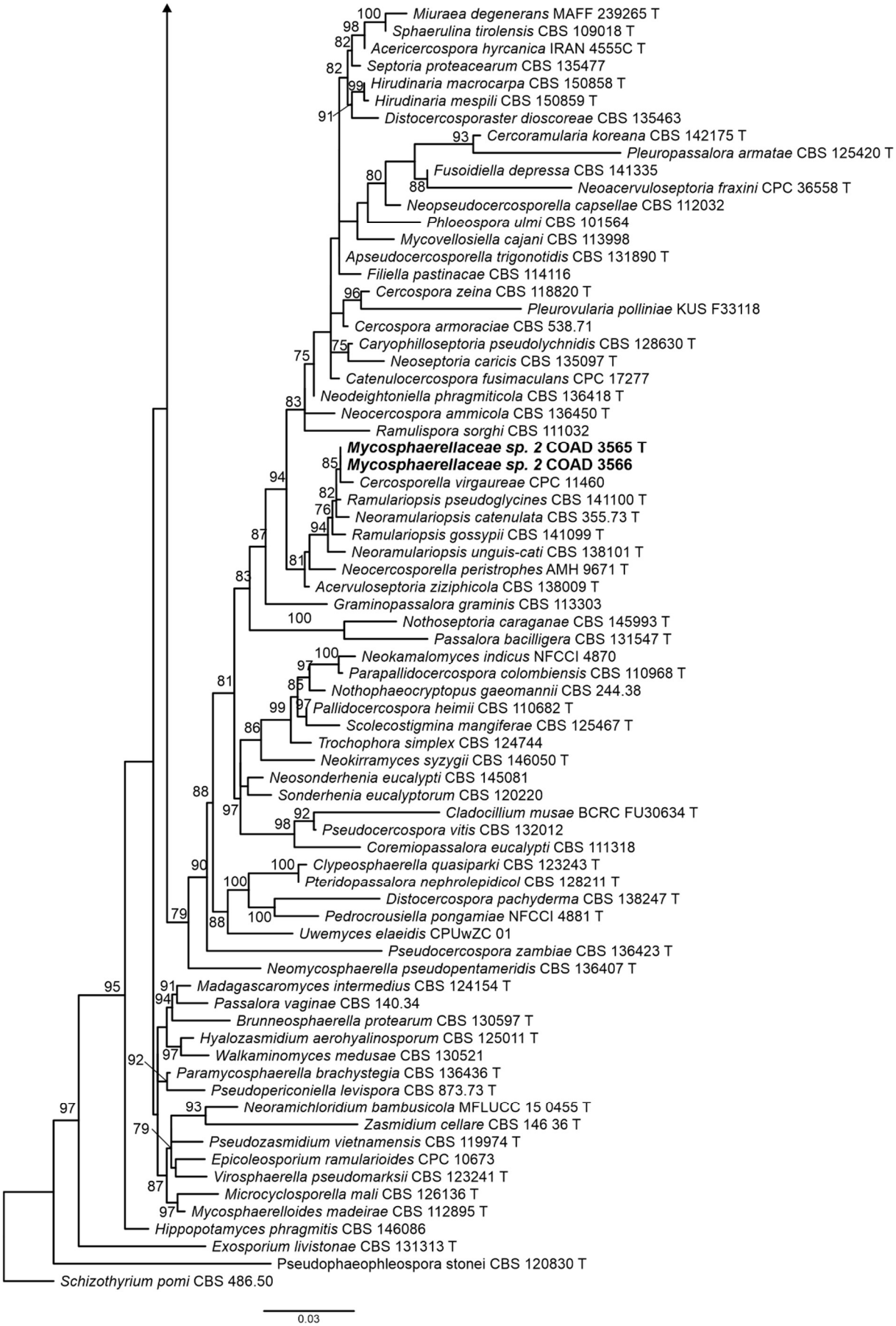
Supplementary Fig. S2.

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Hirudinaria mespili CBS 150859 T
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Trochophora simplex CBS 124744
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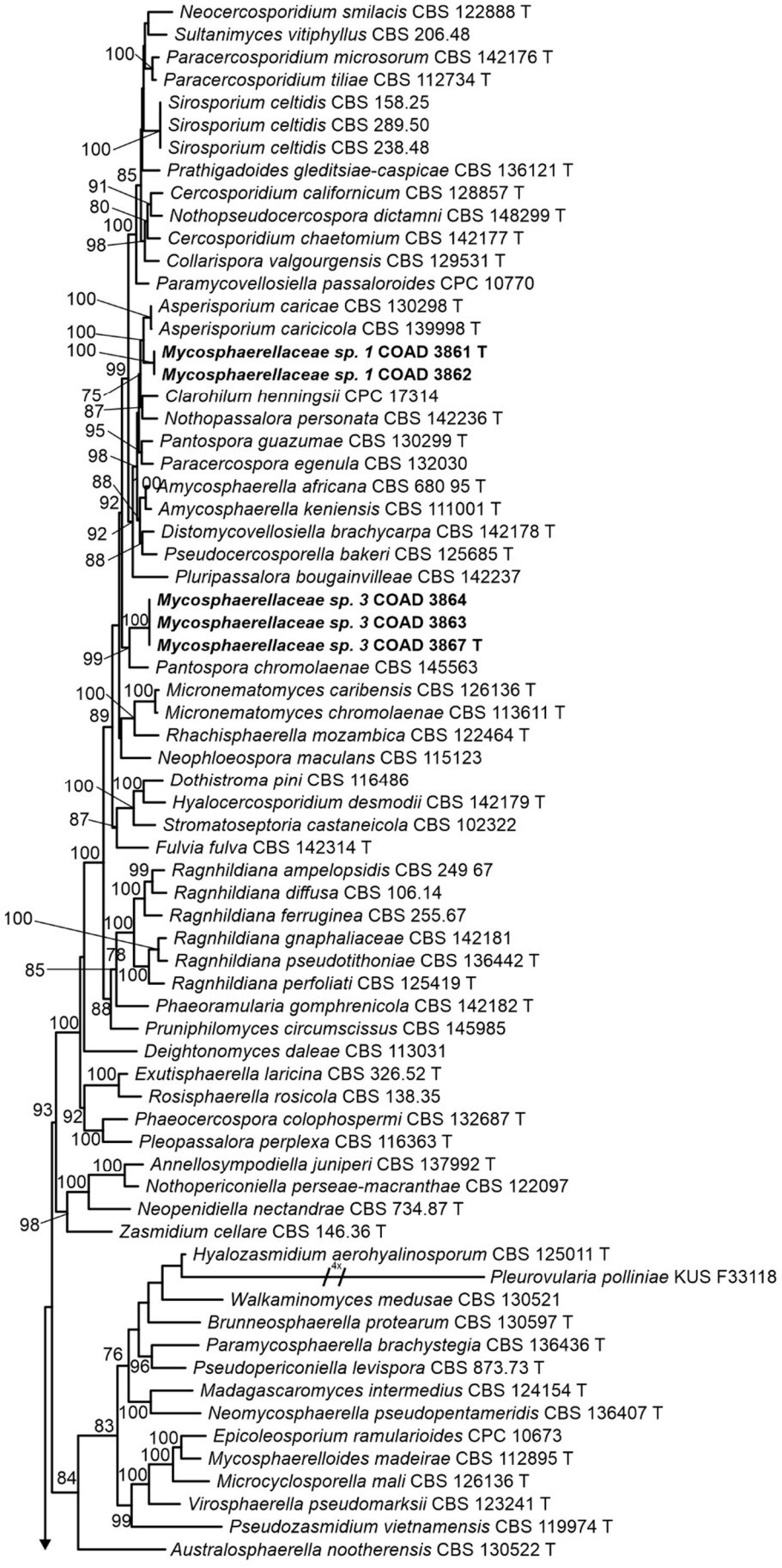
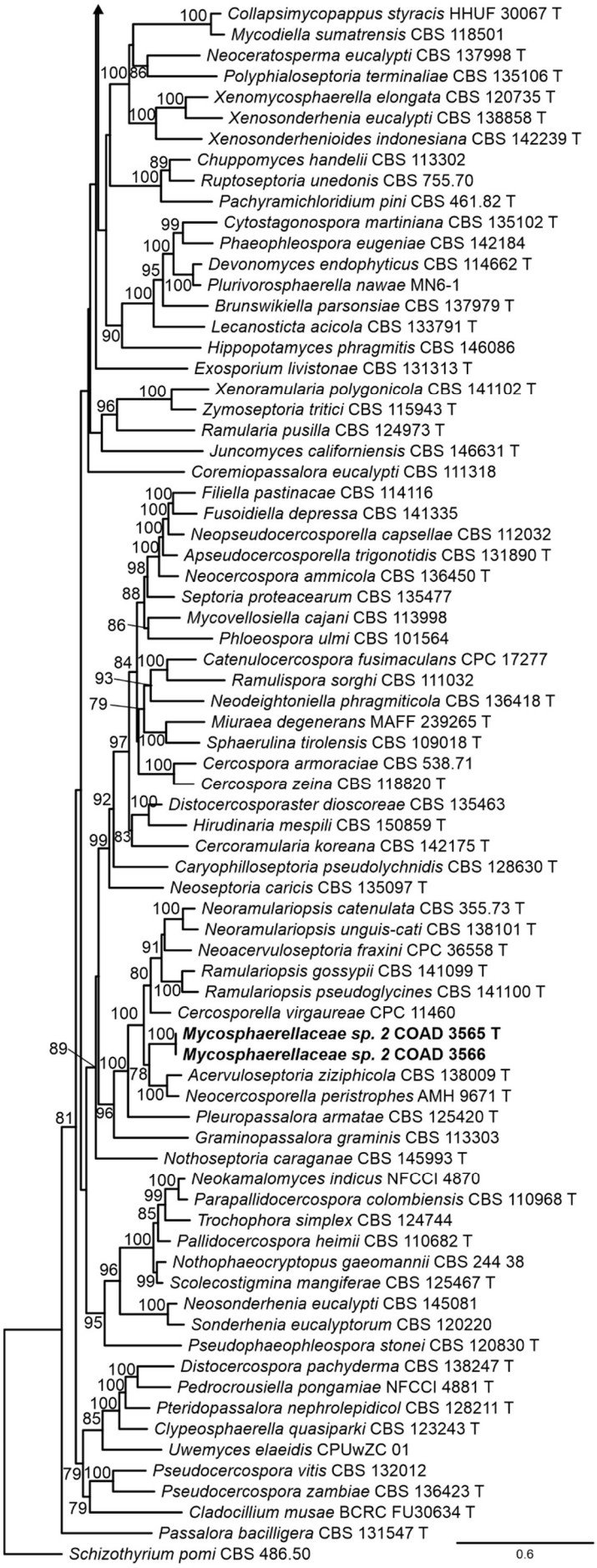
Supplementary Fig. S3.




Collapsimycopappus styracis HHUF 30067 T
Mycodiella sumatrensis CBS 118501
Neoceratosperma eucalypti CBS 137998 T
Polyphialoseptoria terminaliae CBS 135106 T
Xenomycosphaerella elongata CBS 120735 T
Xenosonderhenia eucalypti CBS 138858 T
Xenosonderhenioides indonesiana CBS 142239 T
Chuppomyces handelii CBS 113302
Ruptoseptoria unedonis CBS 755.70
Pachyramichloridium pini CBS 461.82 T
Cytostagonospora martiniana CBS 135102 T
Phaeophleospora eugeniae CBS 142184
Devonomyces endophyticus CBS 114662 T
Plurivorosphaerella nawae MN6-1
Brunswikiella parsonsiae CBS 137979 T
Lecanosticta acicola CBS 133791 T
Hippopotamyces phragmitis CBS 146086
Exosporium livistonae CBS 131313 T
Xenoramularia polygonicola CBS 141102 T
Zymoseptoria tritici CBS 115943 T
Ramularia pusilla CBS 124973 T
Juncomyces californiensis CBS 146631 T
Coremiopassalora eucalypti CBS 111318
Filiella pastinacae CBS 114116
Fusoidiella depressa CBS 141335
Neopseudocercosporella capsellae CBS 112032
Apseudocercosporella trigonotidis CBS 131890 T
Neocercospora ammicola CBS 136450 T
Septoria proteacearum CBS 135477
Mycovellosiella cajani CBS 113998
Phloeospora ulmi CBS 101564
Catenulocercospora fusimaculans CPC 17277
Ramulispora sorghi CBS 111032
Neodeightoniella phragmiticola CBS 136418 T
Miuraea degenerans MAFF 239265 T
Sphaerulina tirolensis CBS 109018 T
Cercospora armoraciae CBS 538.71
Cercospora zeina CBS 118820 T
Distocercosporaster dioscoreae CBS 135463
Hirudinaria mespili CBS 150859 T
Cercoramularia koreana CBS 142175 T
Caryophilloseptoria pseudolychnidis CBS 128630 T
Neoseptoria caricis CBS 135097 T
Neoramulariopsis catenulata CBS 355.73 T
Neoramulariopsis unguis-cati CBS 138101 T
Neoacervuloseptoria fraxini CPC 36558 T
Ramulariopsis gossypii CBS 141099 T
Ramulariopsis pseudoglycines CBS 141100 T
Cercosporella virgaureae CPC 11460
Mycosphaerellaceae sp. 2 COAD 3565 T
Mycosphaerellaceae sp. 2 COAD 3566
Acervuloseptoria ziziphicola CBS 138009 T
Neocercosporella peristrophes AMH 9671 T
Pleuropassalora armatae CBS 125420 T
Graminopassalora graminis CBS 113303
Nothoseptoria caraganae CBS 145993 T
Neokamalomyces indicus NFCCI 4870
Parapallidocercospora colombiensis CBS 110968 T
Trochophora simplex CBS 124744
Pallidocercospora heimii CBS 110682 T
Nothophaeocryptopus gaeomannii CBS 244 38
Scolecostigmina mangiferae CBS 125467 T
Neosonderhenia eucalypti CBS 145081
Sonderhenia eucalyptorum CBS 120220
Pseudophaeophleospora stonei CBS 120830 T
Distocercospora pachyderma CBS 138247 T
Pedrocrousiella pongamiae NFCCI 4881 T
Pteridopassalora nephrolepidicol CBS 128211 T
Clypeosphaerella quasiparki CBS 123243 T
Uwemyces elaeidis CPUwZC 01
Pseudocercospora vitis CBS 132012
Pseudocercospora zambiae CBS 136423 T
Cladocillium musae BCRC FU30634 T
Passalora bacilligera CBS 131547 T
Schizothyrium pomi CBS 486.50
0.6

MATERIAL SUPLEMENTAR

Tabela suplementar 1. Isolados da família *Mycosphaerellaceae*.

Identificação	Código do coletor	Código COAD	Hospedeiro	Local de coleta	Data de coleta
Mycosphaerellaceae sp. 1	PTN21	COAD 3861	*Samanea inopinata*	Viçosa, MG	Jun. 2023
Mycosphaerellaceae sp. 1	PTN22	COAD 3862	*Samanea inopinata*	Viçosa, MG	Jun. 2023
Mycosphaerellaceae sp. 3	PTN47	COAD 3863	*Swietenia macrophylla*	Viçosa, MG	Mar. 2024
Mycosphaerellaceae sp. 3	PTN48	COAD 3864	*Swietenia macrophylla*	Viçosa, MG	Mar. 2024
Mycosphaerellaceae sp. 2	PTN49	COAD 3865	*Erythrina speciosa*	Viçosa, MG	Apr. 2024
Mycosphaerellaceae sp. 2	PTN50	COAD 3866	*Erythrina speciosa*	Viçosa, MG	Apr. 2024
Pseudocercospora variabilis	PTN62		*Libidibia ferrea*	Viçosa, MG	Jun. 2024
Pseudocercospora variabilis	PTN63		*Libidibia ferrea*	Viçosa, MG	Jun. 2024
Mycosphaerellaceae sp. 3	PTN64	COAD 3867	*Swietenia macrophylla*	Viçosa, MG	Jun. 2024
Pleopassalora sp.	PTN65		*Samanea inopinata*	Viçosa, MG	Jun. 2024
Pleopassalora sp.	PTN66		*Samanea inopinata*	Viçosa, MG	Jun. 2024
Cercosporoide	PTN67		*Erythrina verna*	Viçosa, MG	Jul. 2024
Cercosporoide	PTN68		*Erythrina verna*	Viçosa, MG	Jul. 2024
Pseudocercospora sp.	PTN69		*Collaea speciosa*	Domingos Martins, ES	Jul. 2024
Pseudocercospora sp.	PTN70		*Collaea speciosa*	Domingos Martins, ES	Jul. 2024
Passalora sp.	PTN70		*Sapium glandulosum*	Domingos Martins, ES	Jul. 2024